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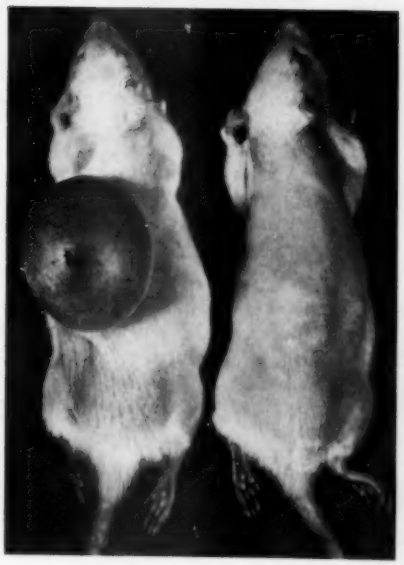

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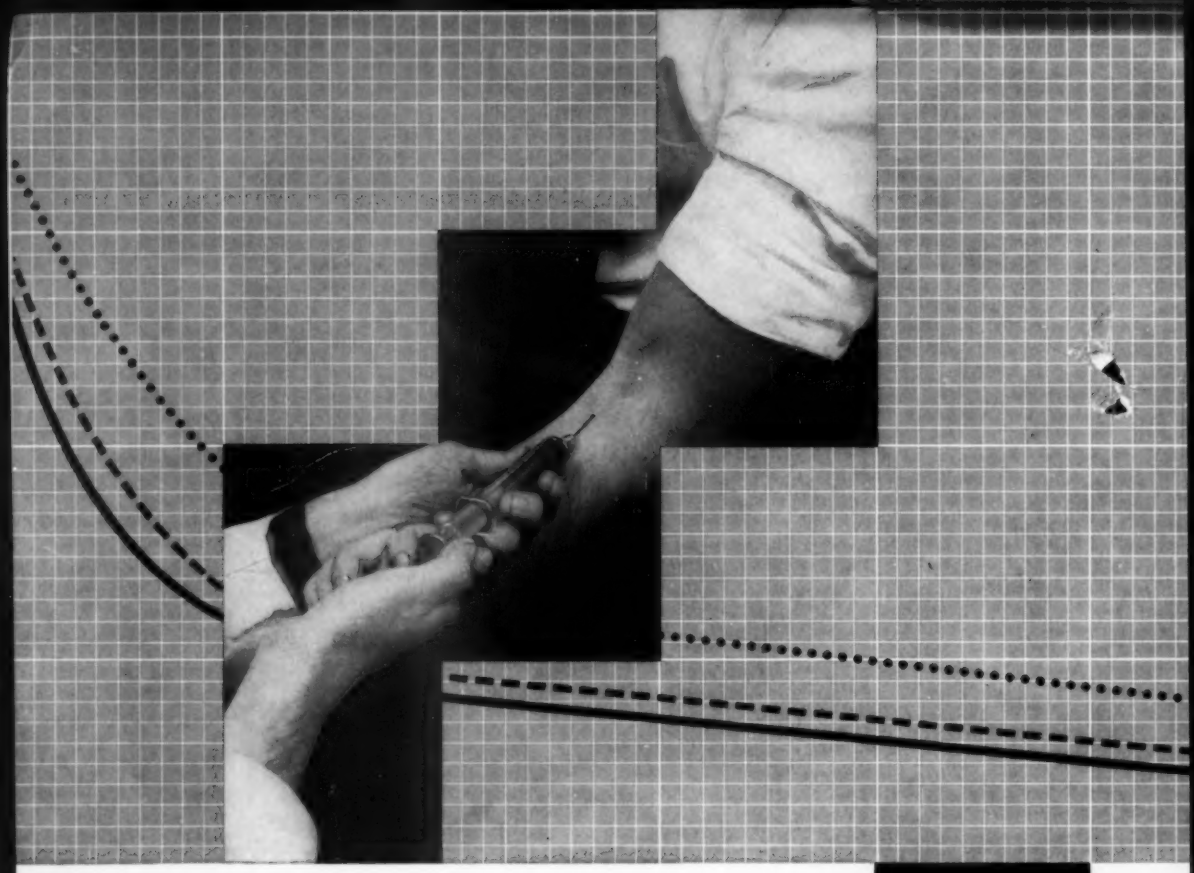

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2. Shapiro, S., and Weiner, M.: J. M. Soc. New Jersey *48*:1 (Jan.) 1951.
3. Shapiro, S., *et al.*: Am. Heart J. *40*:766 (Nov.) 1950.

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# TABLE OF CONTENTS

VOLUME 59 JANUARY 1955 NUMBER 1

## EDITORIALS

PAGE

**Letter from the Editors** ..... 1

## ORIGINAL ARTICLES

**Pathogenesis of Intestinal Ulcers Following Irradiation**
*Nathan B. Friedman, M.D., Los Angeles* ..... 2

**Neuropathy, Cardiopathy, Hemosiderosis, and Testicular Atrophy in Gorilla Gorilla**
*Paul E. Steiner, Ph.D., M.D.; Theodore B. Rasmussen, M.D., and Lester E. Fisher, D.V.M., Chicago* ..... 5

**Experimental Studies on Histogenesis of Blood-Borne Metastases**
*Renato Baserga, M.D., and Umberto Saffiotti, M.D., Chicago, with the Assistance of Joseph Baum, B.S.* ..... 26

**Bronchiolar (Alveolar Cell) Tumors**
*T. C. Laipply, M.D.; J. C. Sherrick, M.D., and W. E. Cape, M.D., Chicago* ..... 35

**Subacute Erosive ("Peptic") Esophagitis**
*Lieutenant Colonel Eddy D. Palmer, Medical Corps, United States Army* ..... 51

**Histochemical Studies of Fibrinoid Substances and Other Abnormal Tissue Proteins**
*Bernard M. Wagner, M.D., Philadelphia*
I. Application of Roque's Chromotrope Stain ..... 58
II. Effect of Fibrinolytic Enzymes ..... 63

**Effect of Vitamin $B_{12}$ on Selenium Poisoning in the Rat**
*R. H. Rigdon, M.D., Galveston, Texas; J. R. Couch, Ph.D., College Station; Doyle Brashear, and R. Taher Qureshi, M.D., Galveston* ..... 66

**Experimental Thyroid Metaplasia in the Rat**
*John H. Van Dyke, Ph.D., Philadelphia* ..... 73

**Histochemical Studies of the Effect of Irradiation of the Placenta**
*Alvan G. Foraker, M.D., Houston, Texas; Sam Wesley Denham, M.D., Jacksonville, Fla., and Dorothy D. Mitchell, A.B., Atlanta* ..... 82

**"Paradoxical" Antinecrotic Effect of Cortisol**
*Hans Selye, M.D., Montreal, Canada* ..... 90

**Leiomyosarcoma of Right Ureter**
*Lieutenant Robert L. Alznauer (MC), U.S.N.R.* ..... 94

**In Vitro Dehydrogenase Activity of Normal and Pathological Lymph Nodes**
*Maurice M. Black, M.D., and Francis D. Speer, M.D., New York, with the Technical Assistance of Jean Watts, B.A.* ..... 100

## SCIENTIFIC EXHIBITS

**Fungous Diseases**
*Emma S. Moss; Albert L. McQuown, and Robert S. Cooke, New Orleans* ..... 105

## REGULAR DEPARTMENTS

**News and Comment** ..... 125

**Books** ..... 127

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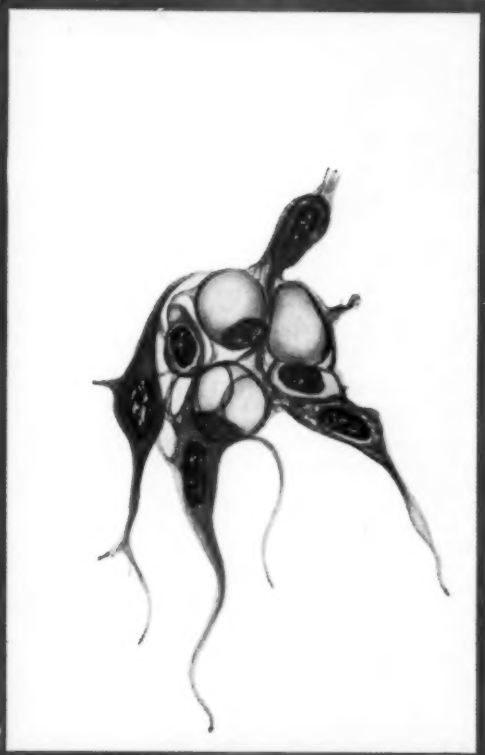

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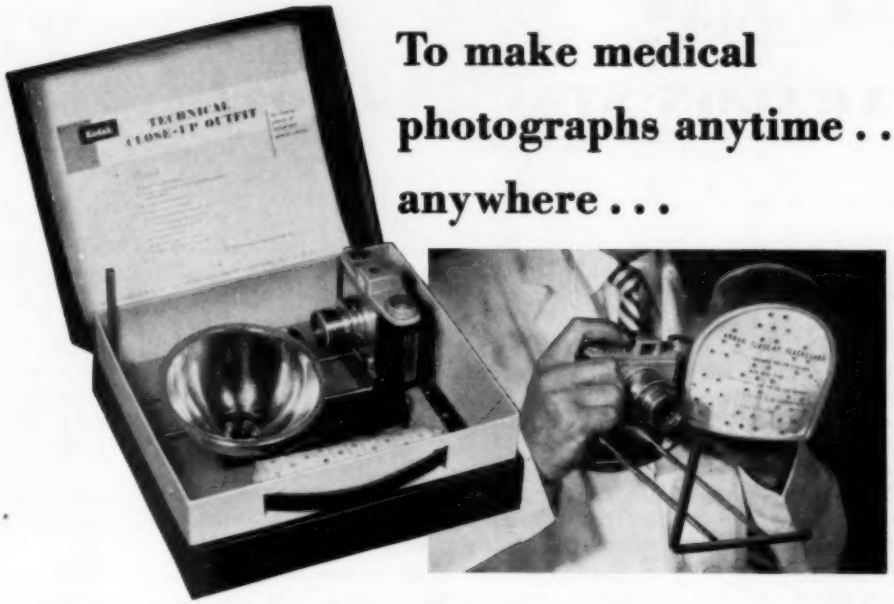


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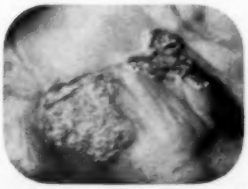

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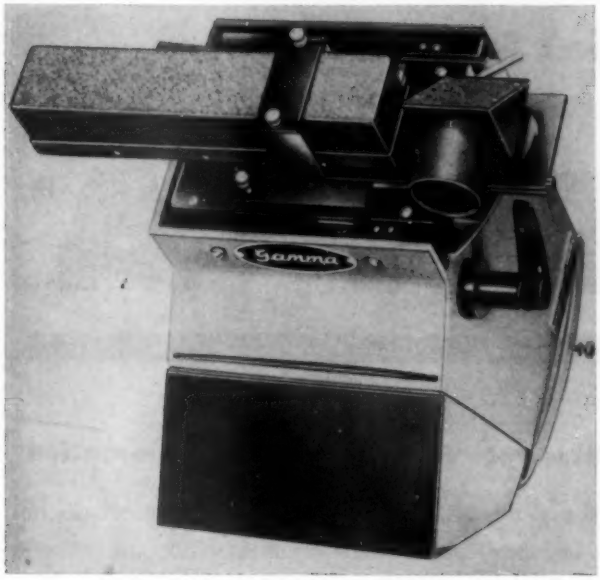


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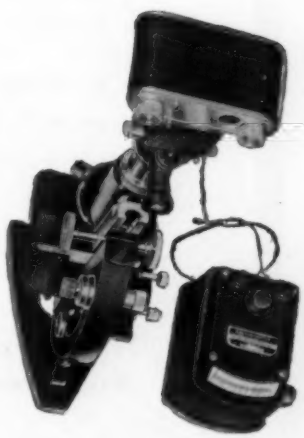

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3. Stollerman, G. H., et al.: Am. J. Med. 15:645 (Nov.) 1953.
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# Editorials

## LETTER FROM THE EDITORS

WE HOPE that you will like the "new look" of the ARCHIVES. The cover has been completely redesigned, and illustrations and space have been rearranged to present a maximum amount of material in the minimum compass compatible with good readability. If you disapprove, the editors accept a full share of responsibility for these changes, because they were consulted freely during the planning. The editors were somewhat disturbed by the fact that this is the second change in the format of the ARCHIVES since its publication began, in 1926. However, they were glad to accede to the new proposals for the cover, provided it contained at least a partial list of the table of contents, a suggestion which was readily accepted by the business management.

It has been felt, in line with the policy of the Editorial Board to attempt to keep abreast of the major interests of all pathologists by such innovations as the Scientific Exhibits, editorials on timely topics, etc., some change in the physical form of the ARCHIVES was in order. The Editorial Board agrees and feels that the changes made are well conceived and executed. The editors will strive to continue to do their part in selecting new, reliable, and informative material for the readers and in presenting it without undue delay.

CHIEF EDITOR

# PATHOGENESIS OF INTESTINAL ULCERS FOLLOWING IRRADIATION

## Effects of Colostomy and Adhesions

NATHAN B. FRIEDMAN, M.D., Los Angeles

ULCERATION is a well-known consequence of irradiation of the gastrointestinal tract.[1] Although most workers have expressed the belief that ulceration reflects the direct effect of ionizing radiation, some studies [2] have indicated that ulcers are produced not by irradiation alone but by the impingement of secondary factors on a surviving, albeit altered, mucous membrane. The opinion has been advanced that focal ulcerative lesions occur at sites of maximal dosage,[3] and it has not been generally recognized that their location probably is determined by secondary factors.

In order to evaluate the roles which the dosage of radiation and the mechanical and bacterial irritation of the fecal stream play in the production of intestinal ulcers, it was decided to test the effects of colostomy and the anchoring of an intestinal segment. The colostomies were done so that the colon might be irradiated in the absence of the fecal stream. The binding of an intestinal segment to the abdominal wall made it possible to irradiate the bowel in such a fashion that the fixed loop of intestine was most heavily treated. This experiment attempted to duplicate the clinical situation in which a loop of bowel adherent to a cervical stump treated with radium or roentgen rays receives heavier irradiation than the remainder of the intestine.

From the Division of Laboratories, Cedars of Lebanon Hospital, and the Department of Pathology, University of Southern California School of Medicine.

Supported in part by the Jane Coffin Childs Fund, the American Cancer Society, and the Atomic Energy Commission. Photographs by Dale Gillette. This work was begun in the Laboratory of Pathology of the New England Deaconess Hospital, Boston, under the direction of Shields Warren, M.D.

### FIRST EXPERIMENT: EFFECT OF ADHESIONS

*Methods.*—Twenty-four rabbits were employed in the first experiment. They were treated with a course of irradiation such as was described in a previous report.[2] A total of 4,000 r was given in daily doses of 400 r. The technical factors were as follows: mechanically rectified x-ray machine with a peak voltage of 140 kv.; 8 ma.; 32.5 cm. target-skin distance, and filtration, 0.25 mm. Cu, 2 mm. Al, and 3 mm. of Celluloid, equivalent to half-value layer of 0.52 mm. Cu. A 10 by 15 cm. anterior abdominal portal was used. The skin dose was 37.6 r per minute. The depth dose was 69% at 4 cm. and 40% at 8 cm. The rabbits, as stretched out during treatment, were approximately 8 to 12 cm. thick.

The animals were divided into two groups of 12 each. The rabbits in the control group were irradiated without prior surgery. In the other 12 rabbits an intestinal loop was made adherent to the anterior abdominal wall. In one group of six a small intestinal loop, and in the other six a segment of large bowel, was fixed to the undersurface of the anterior abdominal wall by skin clips, Cellophane strips, or absorbable surgical (catgut) sutures inserted through the mesentery. Animals were killed and the tissues examined grossly and microscopically approximately every 2 weeks for 10 weeks, and thereafter at about monthly intervals, up to six months after treatment.

*Results.*—In the 12 control rabbits five grossly recognizable ulcerative lesions were found, all in the colon. In the 12 animals which underwent surgery six ulcers were identified. Five lesions were in the colon, three in a nonadherent loop, and two in the loop fixed to the anterior abdominal wall; in one animal there was an ulcer in a nonadherent small intestinal loop. There were, therefore, twice as many ulcers in nonadherent loops as in those which had been fixed. The characteristic microscopic lesions usually seen after irradiation of the intestine were present throughout the bowel, and in four instances there was slightly more diffuse damage in the mucosa of the adherent loop than in control segments.

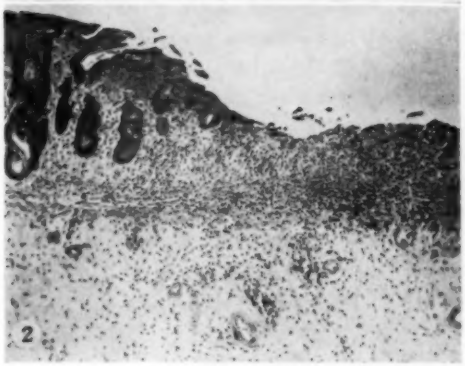


Fig. 1.—Intestinal ulcer following implantation of a 10 mc. radon seed into the psoas muscle of a normal rat; reduced 32% from mag. × 20.

Fig. 2.—Intestinal ulcer following implantation of a 10 mc. radon seed into the psoas muscle of a normal rat; reduced 32% from mag. × 180.

Incidence of ulcers in adherent and nonadherent intestinal loops of rabbits after irradiation was as follows:

| | Rabbits with Adherent Loops | Control Rabbits |
|---|---|---|
| Ulcers in adherent loop..... | 2 | .. |
| Ulcers in nonadherent loop.... | 4 | 5 |
| No ulcers ..... | 6 | 7 |
| Total ..... | 12 | 12 |

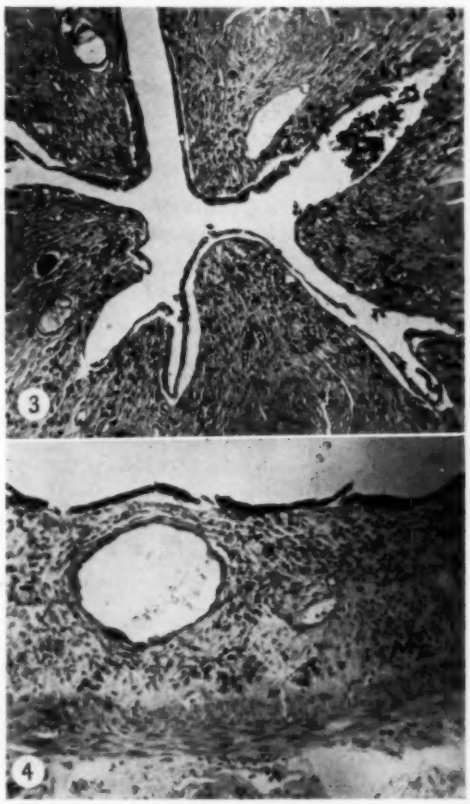
SECOND EXPERIMENT: EFFECT OF COLOSTOMY

*Methods.*—Enterostomies near the ileocecal valve were performed in a number of rabbits, but the animals did not survive the operation more than a month. They became lethargic, lost weight, and paresis of the hindlegs appeared. The short circuiting of the cecal appendage was held responsible. It was found that rats survived colostomy in good health for weeks; they therefore were employed in place of rabbits for this experiment. Gold seeds containing 10 to 15 mc. of radon were inserted through an anterior abdominal incision into the psoas muscle alongside the descending colon in 50 rats. One-third of the group were subjected to colostomy at the time that irradiation was begun; one-third had had colostomy performed a week or two before, and one-third had no operation. The animals were killed and tissues examined grossly and microscopically at approximately weekly intervals for one month after irradiation.

*Results.*—Atrophy, atypical metaplasia, and other well-known alterations produced by irradiation of the intestine were present in both control and experimental animals. Ulcerative lesions of the bowel (Figs. 1 and 2) were found only in those animals which

Fig. 3.—Colon of rat which underwent colostomy prior to implantation of 15 mc. radon seed into psoas muscle. Nonfunctioning segment shows extreme mucosal atrophy, but surface epithelium is present, and there is no ulceration or inflammation. Reduced 28% from mag. × 180.

Fig. 4.—Colon of rat which underwent colostomy prior to implantation of 15 mc. radon seed into psoas muscle. Nonfunctioning segment shows almost complete loss of glands, but surface epithelium persists. Reduced 28% from mag. × 180.



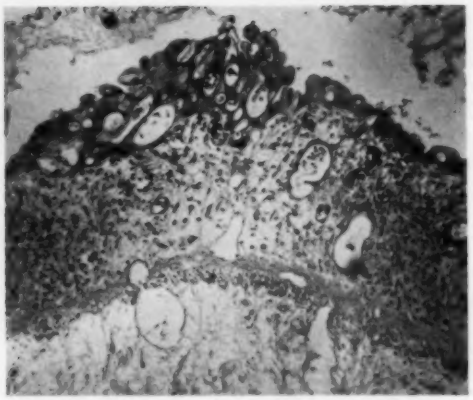


Fig. 5.—Colon of rat which underwent colostomy prior to implantation of 10 mc. radon seed into psoas muscle. Nonfunctioning segment shows persistence of bizarre surface epithelial layer despite severe radiation reaction. There is no ulceration. Reduced 39% from mag. × 180.

did not have colostomies. The ulcers were usually located on the side of the intestine nearest the radon seed. Ulceration did not take place in the rats with colostomies. Despite extreme atrophy of the glandular epithelium, the surface epithelium was preserved in these animals (Figs. 3 and 4), although it exhibited fantastic alterations (Fig. 5).

## COMMENT

Doses of radiation sufficient to produce intestinal ulcers failed to result in ulceration when the fecal stream was deflected by means of colostomy. Ulcers did not always develop at points of maximal irradiation. The results of the experiment indicate that the diffuse intestinal injury produced by irradiation merely sets the stage for secondary damage and that fecal mechanical and bacterial action is probably responsible for ulceration. Irradiated mucosa cannot replace itself successfully or repair the minor injuries normally produced by the passage of feces. The observation that ulcers are less frequently encountered after irradiation of a gastric Pavlov pouch than after treatment of the intact stomach [4] supports this view.

Irradiation ulcers, and possibly other ulcerative lesions of the gastrointestinal tract, result from the interaction of multiple factors, one of which appears to be the passage of intestinal contents. Although "peptic" ulcers, the lesions of amebiasis, uremic colitis, and irradiation ulcers are obviously different entities, these lesions of the gastrointestinal tract also have certain general similarities. No matter what mechanism inflicts the initial damage, it seems evident that whether or not ulceration occurs depends on secondary factors, in particular, the effects of the movement of the fecal stream. The gastrointestinal tract, like other tissues and organs, reacts to injury in a pattern determined not by the nature of the injury but by its own structural and functional organization.

## SUMMARY

Adhesions do not favor the localization of irradiation ulcers in fixed intestinal loops. Colostomy protects irradiated intestinal mucosa from the development of ulcers. Ulceration following irradiation results from the action of secondary factors on a damaged intestinal mucous membrane. The reaction pattern of damaged intestine is determined more by the function and structure of the bowel than by the nature of the injury.

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# NEUROPATHY, CARDIOPATHY, HEMOSIDEROSIS, AND TESTICULAR ATROPHY IN GORILLA GORILLA

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BUSHMAN, the giant lowland gorilla at Chicago's Lincoln Park Zoo, widely known to thousands of admirers, succumbed to a seven-month illness characterized predominantly by pareses of the legs and one arm. He had attained one of the largest sizes (550 lb. [250 kg.] maximum) and what was at that time the greatest age (22 years) of any gorilla in captivity except one, which was said to be about 3 years older. The life span of wild gorillas is not known. It was thought that Bushman might be considered biologically elderly, but this age is now believed, on the basis of the present findings, to be far short of senility. No male in captivity is known to have reached sexual maturity, although the other three species of anthropoid apes have reproduced under similar living conditions.* It is certain from the necropsy findings that Bushman also was infertile. At least three other gorillas are known to have had pareses or paralyses of the extremities, but no clinical or pathological studies on them are available. One such case, that of Janet Penserosa, of the New York Zoological Society, was mentioned by Kennard and Willner,[3] by Hooton,[4] and by Schultz.[5] Another gorilla (Massa, now in the Philadelphia Zoo) had partial paralysis of the lower extremities but recovered.[4] A third (Lady Congo)† was seen at the Brookfield Zoological Gardens, Chicago.‡ This challenging combination in captivity of shortened life, infertility, and paresis and paralysis of the extremities, which is sometimes fatal, poses a problem which, we believe, has been elucidated by the necropsy findings in Bushman. These are herewith presented, together with the few pertinent observations reported in the literature.

In brief, it is believed that the pathological findings indicate a nutritional disorder, chiefly in the nature of dry chronic beriberi, but probably combined with other deficiencies in the vitamin B group and possibly in vitamin E. The most striking pathological changes consisted of retrogressive lesions in the nervous and muscular systems, heart and blood vessels, testes, and the liver and spleen. That these lesions should have developed despite strenuous efforts to maintain a diet adequate according to the best available knowledge indicates again the complexity of nutritional problems in the different species and suggests that the gorilla may have some unique species nutritional requirements.

This idea is strengthened by the sharply restricted natural habitat of the gorilla, which has been a puzzle to primatologists. In historical times, their range has always been limited to two areas in equatorial Africa. One centers in what is now the Cameroons, where the lowland species (Gorilla gorilla) is found. The other area is in the eastern Belgian Congo, where the mountain gorilla (Gorilla beringei) is at home. Both areas are hot and wet, and they have a luxuriant vegetation, on which the gorilla is believed to be largely nourished. Although the intervening country, some 700 miles in extent, outwardly appears similar in character, it

From the Departments of Pathology and Neurosurgery, the University of Chicago (Drs. Steiner and Rasmussen), and the Lincoln Park Zoological Gardens (Dr. Fisher).

* References 1 and 2.

† Freeman, S.: Personal communication to the authors.

‡ According to newspaper accounts, a gorilla in Florida had paresis or paralysis of the extremities in February, 1954.

is not known to have ever supported the gorilla, although a smaller anthropoid ape, the chimpanzee, occupies this entire range, including that of the gorilla. We suggest that some special nutritional element, essential for the gorilla but not for the chimpanzee, may be missing or inadequate in this intermediate area.

Another point of importance in this report is the great need, which Yerkes [2] and Hediger [6] have recently emphasized, for preserving this species. Thus Yerkes has written:

> Of all the types of animals I have observed naturalistically or used in experiments during almost sixty years of work as psychobiologist, none has so stirred my curiosity and suggested so many questions as the gorilla. Whether we deem it success or failure, it is one of Nature's impressive experiments in primate evolution, which probably nears its end. For reasons which will appear, this is a plea for more and better gorilla study—at once more inclusive and intensive, more persistent and imaginative, more critical and enlightening.

Yerkes concluded that only a few hundred examples of G. beringei and a few thousand of G. gorilla are alive today, the latter rapidly decreasing. If adequate conditions could be found for them to reproduce in captivity, this important subhuman primate might be saved for future studies. Hediger classified the gorilla among the "nonbreeders" in captivity.[6] He did not know whether failure to reproduce was due to interference with mating ceremonial or to disease.

Despite the urgent need to understand and correct the disease problem, including those of sterility and paralyses, reports of only two adequate postmortem studies have come to our attention. One is the excellent monograph by Koch [7] on Bobby, the Berlin gorilla, which died in 1935 of perforated appendicitis, at the age of 10½ years. The other autopsy was by McKenney, Traum, and Bonestell [8] on M'Bongo, of the San Diego Zoo, which succumbed to acute coccidioidomycosis. Although the causes of death were different from that of Bushman, certain of the incidental pathological findings have been of great aid in interpreting various aspects of the present changes. Nephritis and infections were given as the other causes of death in gorillas by Hooton,[4] who gave no details. The same author also stated that Congo, which was so intensively studied by Yerkes, died of acidosis secondary to ileocolitis. It is obvious that further pathological studies are needed. Because there is a paucity of information on the normal visceral anatomy and histology of the gorilla, a separate report has been made of those aspects.[9]

## CASE HISTORY

Bushman was born in the Cameroons, French Equatorial Africa, in February or March, 1928. When he was brought to the American Presbyterian Mission at Yaoundé, by Dr. W. C. Johnston, he weighed 8 lb. (3,630 gm.). No major infant diseases were reported, and he grew at a rate of about 1 lb. a month. He arrived at the Lincoln Park Zoo in Chicago via an American animal dealer, J. L. Buck, on Aug. 18, 1930. He then weighed 38 lb. (17.2 kg.). The weights during the first few years were as follows:

| Date | Weight, Lb. |
|---|---|
| August 18, 1930 | 38 |
| June 4, 1931 | 55 |
| May 15, 1932 | 71½ |
| December, 1932 | 76 |
| May, 1933 | 86 |
| September, 1933 | 95 |
| February, 1934 | 105½ |

In November, 1949, he weighed approximately 550 lb. (249.4 kg.) and stood 6 ft. 2 in. (188 cm.) tall.

His life from a medical standpoint was rather uneventful. The attending veterinarian for many years was Dr. W. A. Young. When Bushman was approximately 5 years old, he suffered a rather severe attack of an "arthritic-like" condition, for which he was treated primarily with boiled grapefruit juice and long exposures to sunshine.

Bushman daily ate about 22 lb. of fresh fruit, vegetables, and whole wheat raisin bread, in addition to 3 qts. of milk. Fresh water was available, and multiple vitamins were added to the diet. At one time, when he appeared excessively obese, the cream was removed from his milk. He never ate meat. Digestive disturbances were readily controlled by dietary changes. His keeper considered a soft stool to be normal and endeavored to keep it so throughout the animal's life.

In 1944 Bushman injured the left foot while running and sliding on a wooden cage floor. Ever since that time he had minor recurring ulceration and erosion on the bottom and side of the foot, for which he compensated by altering his gait. Since

his early years, he had never been handled directly for medical purposes, and was treated only by indirect means.

On May 23, 1950, Bushman showed signs of more pronounced lameness. On June 3 he fell down while trying to climb into his runway and on June 6 was much weaker than at any time previously in his life. Primary symptoms were anorexia, dyspnea, extreme flexion of the toes and feet, easy fatigability, and locomotion almost entirely by using the arms. A urinalysis made at that time was normal. Treatment consisted of digitalis, pentylenetetrazole (Metrazol), and other supportive therapy, including the addition of vitamin B complex to his food and fluids. He showed slow but steady improvement and on June 12 appeared out of danger. His legs and right hand, however, remained partially paralyzed and imperfectly coordinated.

On June 27 a group of medical men from the University of Chicago visited Bushman and from observation offered the following tentative diagnosis: a combination of senile changes, some arteriosclerosis, and peripheral neuritis.

No major changes occurred until Dec. 15, when he showed signs of recurring anorexia and weakness. His condition steadily became worse, and he showed no improvement from what little medication he would take. He died on Jan. 1, 1951. He had lost about 50 lb. (22.7 kg.) from his maximum weight.

## POSTMORTEM EXAMINATION

The necropsy was begun three hours after death and lasted eight hours. Because of the large size, thick pelt, and abundant adipose tissue, body heat was lost slowly and microscopic sections of some organs show postmortem changes. After skinning, the brain and spinal cord were removed first; so these tissues are best preserved. For the sake of brevity, the gross and microscopic descriptions are combined and confined to what is considered essential for a presentation of the abnormal findings. The present description omits most of the normal anatomical details, for which the interested reader is referred to another paper.[9]

*External Appearance.*—The body was that of a large male gorilla, weighing about 500 lb. (226.8 kg.), in which rigor mortis was present only in the jaw; body heat was still present. The general appearance was that shown in Figure 1. The head and trunk were large in proportion to the extremities, and the upper extremities were larger and longer than the lower. The crest was well developed; the neck was short and thick; the lower jaw was large, and the forehead was of the receding type. The thighs and legs were relatively short; the left was smaller than the right, particularly in the region of the calf, where actual atrophy was visible. The left foot was partly everted, and it showed evidence of wear and deformity along the lateral margins (Fig. 2). The left toes were turned down and inward. The distal end of the left fifth toe was missing and the site scarred. On the plantar surface of the left heel was an oval, sharp, deep ulcer, measuring 5 by 4 cm. It had overhanging skin margins, steep walls, and a clean base, composed of yellowish fat and connective tissue, which formed a layer about 5 mm. thick on the underlying calcaneus, which itself was not exposed. There was no discharge and little odor from this ulcer.

The configuration of the head can be seen in the photograph. There was no discharge from eyes, nose, ears, mouth, or anus. The shoulders and arms were large. The thorax was huge, and it widened still more at the bottom where it connected with the abdomen. This abdomen, protuberant when the animal was in the squatting or quadrupedal position and nearly flat in the erect position, was large, soft, and slightly protuberant at autopsy. The abdomen narrowed sharply at its lower end to a relatively small pelvis (Fig. 1).

The skin was rough, dry, and dark grayish-black. It was covered by a thick growth of long (up to 4 cm.), nearly black, coarse hair. In each pectoral region was a small, flattish nipple. The penis and scrotum appeared small, flabby, and rudimentary. The thick pelt was firm, semirigid, and from 1 to 1.8 cm. thick. Its removal exposed an abundant but incomplete layer of bright yellow subcutaneous adipose tissue, which covered most of the muscles. The exposed muscles, in sharp contrast, were strikingly dark reddish-brown. The laryngeal air sacs were examined at several places in the neck, subclavicular region, and anterior axilla. They were com-

Fig. 1.—Photograph of Bushman in bipedal position. The large arms, configuration of the trunk, terminal ends of the laryngeal air sacs in the axillae, short legs, and small external genitalia are shown. The leg atrophy was not seen at this time.

plex in configuration, normally empty, moist, pale whitish-gray, smooth, and glistening. Externally, the lowest extensions of these air sacs appear in Figure 1 as the bulging areas in the anterior axillary folds. There was no visible evidence for the loss of 50 lb. reported to have occurred during the last six months other, perhaps, than in the deep yellow color of the subcutaneous adipose tissue.

*Serous Body Cavities.*—The anterior abdominal wall, after the pelt was removed, was only about 1.5 cm. thick. These muscles appeared relatively poorly developed and inconspicuous in their adjoining fat. The peritoneum was pale, smooth, and glistening, and there was no free fluid. Numerous (about 50) bands of dense fibrous adhesions, averaging about 1 cm. in diameter, were found in the anterior part of the peritoneal cavity. Most of these adhesions were between the omentum and the abdominal wall, or between the colon and either the omentum or the abdominal wall. No adhesions were found in the pelvis, in the lesser peritoneal cavity, about the liver or stomach, or in the posterior part of the greater peritoneal cavity. The small intestine was normally free.

When the abdomen was opened, the presenting part was the very obese omentum. It weighed 3,700 gm. After it had been re-

Fig. 2.—Photograph of left foot, showing the chronic ulcer on the heel, the missing fifth toe, and wear on the lateral margin.

moved, the most conspicuous viscus was the very large cecum and ascending colon. (It resembled that shown by Koch in Figure 2 in his article on Bobby, the Berlin gorilla.) The other abdominal organs appeared relatively normal. Fat was also abundant in the mesentery (weight 1,650 gm.) and in many subperitoneal and retroperitoneal sites (Fig. 3). This internal fat was everywhere pale whitish-yellow, in contrast to the bright yellow subcutaneous adipose tissue.

The pleural surfaces were pale, smooth, and glistening. The spaces contained no free fluid and no adhesions. The large pericardial sac contained about 50 cc. of clear, pale, straw-colored fluid. The surfaces were pale, smooth, and glistening.

*Respiratory Tract.*—The larynx was larger than in man. The vocal cords were distinct, and a good space was present between them. When the larynx was opened, a puffy, pitting edema of each laryngeal wall from the vocal cords down to the trachea was visible. The mucosa was pale and smooth, and there was no excess secretion or any foreign body. The tracheal mucosa showed a slight hyperemia. This reddening was progressively more conspicuous as one passed from the main to the smaller bronchi. Frothy, slightly blood-tinged fluid welled up into the main bronchi when the lungs were compressed.

The right lung weighed 1,110 gm., the left 850 gm. The left lung was bilobed, but the right had four lobes, the usual right lower lobe being almost completely subdivided into two. After removal, the lungs were not quite fully inflated, but their size was approximately that found in a large man. They were relatively shorter but broader than human lungs. They had a slight amount of anthracotic pigment, which appeared to be about average for a 22-year-old Chicago resident. Anteriorly the lungs were light and fluffy but slightly reddened. The posterior two-thirds of the left lung and the posterior halves of the upper and lower lobes of the right lung were dark purplish-red, firm, pitting, and almost noncrepitant. The transition from pale to dark lung was rather abrupt.

Fig. 3.—Photograph of the partly eviscerated abdominal cavity, showing the abundant, if not excessive, fat, relatively flat diaphragm, and lobulated liver. The large larynx is seen immediately above the ends of the clavicles in the short neck.

The cut su ces showed a slight hyperemia anteriorly and a severe hyperemia and edema posteriorly, with a transition zone between. Frothy, bloody fluid gushed from the smaller bronchi. There was no evidence of pneumonic consolidation and none of tuberculosis.

Microscopic examination disclosed minimal anthracosis. A few leucocytes, with fluid, mucus, and bacteria, were present in some of the bronchi. Anteriorly a little edema fluid and occasional alveolar necrophages were seen in some alveoli; posteriorly there was a severe edema, which was hemorrhagic in some places in alveoli, and also much interstitial edema (Fig. 4). Within the layers of the fibrous pleura at a point where no adhesions had been present were found a few adipose tissue cells. The tracheal mucosa showed much postmortem change but no important antemortem abnormality.

*Cardiovascular System.*—The heart appeared broad and relatively short. This configuration was due in part to hypertrophy and dilatation of the right chambers. As viewed from the front, more than half the surface was made up of right heart. The epi-

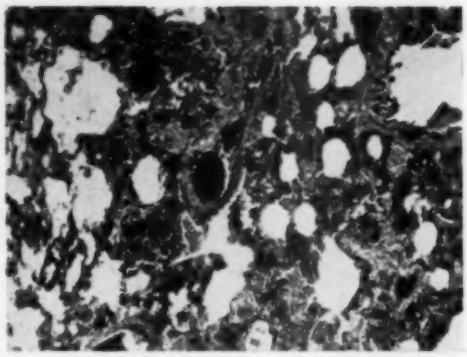
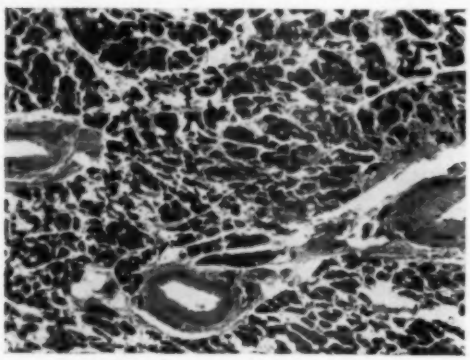


Fig. 4.—Lung. Photomicrograph of the posterior part, showing hemorrhagic edema; hematoxylin and eosin; reduced ⅓ from mag. × 53.

cardium was smooth but very obese. It was estimated to contain about 50 gm. of adipose tissue in a layer up to 12 mm. thick. An area of white fibrous epicardial thickening about 2 by 3 cm. was present on the anterior surface of the right ventricle near the septum. The emptied heart weighed 805 gm. The chambers contained only fluid blood. The myocardium was soft, flabby, and pale brownish-red but mottled by slightly darker areas. The fibrous tissue about the blood vessels appeared conspicuous. Indistinct small grayish depressed areas, evident in the myocardium, resembled fibrous scars. Despite dilatation, the myocardium of the right ventricle measured 6 to 10 mm. in thickness and that of the left ventricle varied from 8 to 18 mm.,

Fig. 5.—Heart. Photomicrograph of the left ventricle. One of three arteries shows sclerosis. There is also hypertrophy of some muscle fibers and interstitial edema. Hematoxylin and eosin; reduced ⅓ from mag. × 70.



exclusive of the fat. The endocardium of the chambers and valves was thin and smooth. The valves measured as follows: tricuspid, 15.0 cm.; pulmonary, 11.2 cm.; mitral, 13.0 cm., and aortic, 9.2 cm. The base of one aortic leaflet showed a slight fibrous thickening. The coronary arteries showed no sclerosis. The left coronary had no true circumflex branch, but only a twig that coursed obliquely across and down the upper anterior surface of the left ventricle. The right coronary artery was about half as large as the left, which, in turn, was about the size of the corresponding artery in man. The coronary arteries appeared small in proportion to the volume of myocardium.

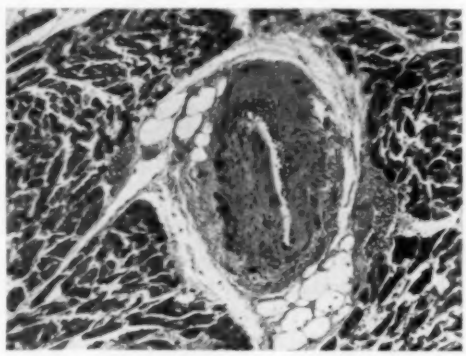


Fig. 6.—Heart. Photomicrograph showing another focally sclerotic and partly hyalinized artery in the left ventricular myocardium. This section shows also hypertrophy and fragmentation of some muscle fibers and infiltration by adipose tissue. Hematoxylin and eosin; reduced ⅓ from mag. × 100.

The aorta was elastic and free of any visible intimal abnormality, as were its main branches and all other blood vessels encountered during the examination. At the level of the diaphragm it was only 5.2 cm. in circumference and at the bifurcation 4.9 cm. The pulmonary artery and its main branches, the superior and inferior vena cavae, the portal system, and other vessels showed no grossly recognizable abnormality.

Microscopically the heart showed many abnormalities. Frozen sections stained for fat revealed fine droplet, sudanophilic material present in practically every myocardial fiber, but with considerable variation in amount

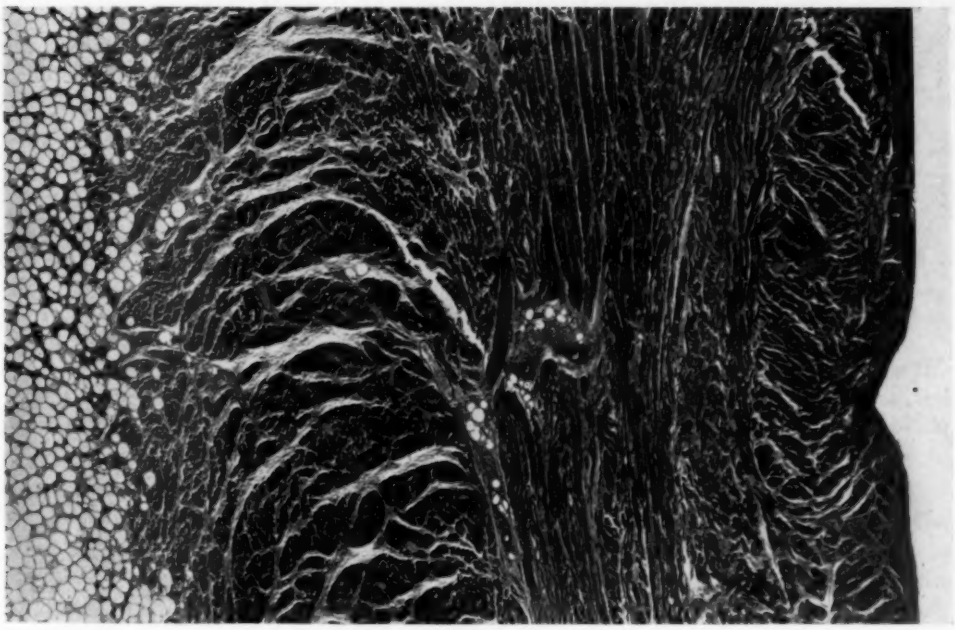
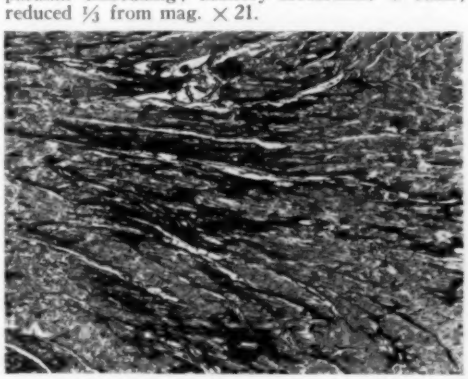


Fig. 7.—Heart. Photomicrograph of the right ventricle showing interstitial edema and fibrosis, hypertrophy, and adipose tissue invasion; celloidin embedding; hematoxylin and eosin; reduced ⅓ from mag. × 23.

from cell to cell. In most sections much fragmentation of fibers was evident (Figs. 5 and 6). Both ventricles showed a considerable degree of variation in size of fibers, the larger of which were definitely hypertrophic. Such enlarged fibers had large nuclei with squared ends (Figs. 5 and 6). There were widespread, slight to severe increases in interstitial connective tissue and some actual fibrous scars (Figs. 7 and 8), not disposed about blood vessels. Most of the sections (including those with celloidin embedding) exhibited a mild to severe amount of interstitial edema (Figs. 5, 6, and 7). The main coronary arteries and most of their branches appeared normal. About 10% of the small myocardial arteries showed sclerosis, consisting mostly of a slight thickening, with fibrosis and sometimes glossy hyalinization, involving chiefly the media but sometimes all layers (Figs. 5 and 6). In severe lesions the internal elastic membrane had degenerated. The epicardial fat had extensively infiltrated the myocardium of both ventricles (Fig. 6), occasionally reaching nearly to the endocardium (Fig. 7).

Microscopic examination of blood vessels in many parts of the body showed sclerotic changes in only the heart and nervous system. In addition, the aorta, which grossly had appeared to be normal, showed areas of thickening of the intima (Fig. 9), and a similar plaque was seen in the celiac artery.

Fig. 8.—Heart. Photomicrograph of the left ventricle showing focal and diffuse myofibrosis and fragmentation and hypertrophy of some fibers; paraffin embedding; Mallory-azocarmine G stain; reduced ⅓ from mag. × 21.



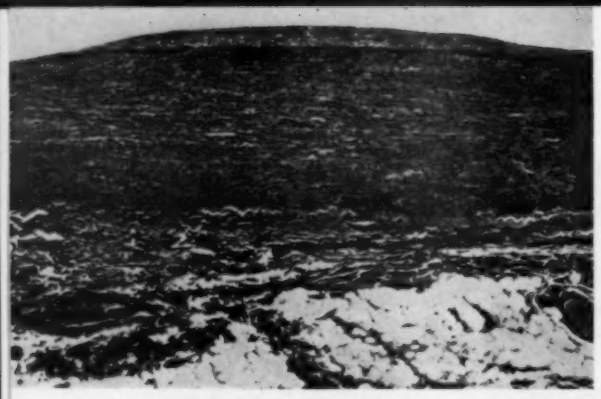


Fig. 9.—Aorta. Photomicrograph of the lower abdominal aorta with an area of intimal fibrous thickening; hematoxylin and eosin; reduced ⅓ from mag. × 30.

Grossly these areas were white and not yellow, and microscopically they were eosinophilic and not pale or foamy. They appeared to be chiefly fibrous.

*Alimentary Tract.*—The mouth showed mild gingivitis, severe fetor oris, and caries of one left lower molar. The mucosa was pale. The lymphoid tissue of the tonsils and elsewhere appeared normally conspicuous. The major salivary glands did not appear abnormal. The large esophagus had a smooth, grayish mucosa except for a few small reddish erosions near the lower end. The stomach was large and thick-walled. It contained several hundred cubic centimeters of thin, bile-stained fluid and a little mucus. The mucosa showed a few petechial hemorrhages. The duodenum, jejunum, and ileum were large, but essentially normal. They contained a small amount of brownish, thick fluid, and the mucosa showed scattered areas of slight hyperemia. The appendix was long, slender, and grossly normal. The colon contained semisolid, yellow feces. Its mucosa showed areas of hyperemia. Solitary lymphoid follicles were conspicuous in the descending and sigmoid regions. No parasites or ulcers were seen in the alimentary tract. The pancreas is described with the endocrine system.

Microscopically a large salivary gland appeared essentially normal. The esophageal mucosa showed erosions in the lower third. The cardia, fundus, and pylorus of the stomach were not remarkable except for adipose infiltration into the submucosa and some postmortem changes in the mucosa. The duodenum was not remarkable. The appendiceal lymphoid tissue was scanty, and a great proliferation of neurofibromatous tissue was present in its mucosa and submucosa. The colon was not abnormal except for a large amount of fat in the submucosa and some postmortem autolysis.

*Liver and Biliary Tract.*—The liver was reddish-brown and weighed 2,200 gm. It exhibited one small lobe and three main lobes; caudate and quadrate lobes were not developed. The capsule was thin, tense, smooth, and almost transparent. The cut surfaces bulged slightly, revealing a uniform brown surface, on which small liver lobules were poorly seen. The liver was flabby and not perceptibly hyperemic. The large gall bladder was partly embedded in the largest lobe of the liver at the expected site. It contained about 40 cc. of thin, clear, amber bile. Its wall was thin, and its mucosa was studded by projecting yellow nodules, averaging about 1 mm. in diameter. The extrahepatic bile ducts were not visibly abnormal.

Microscopically the liver showed small lobules, with atrophic cords and widened, blood-containing sinuses (Fig. 10). The congestion was greatest about the central veins. A frozen section stained for fat showed a small amount of the small droplet type rather evenly dispersed through the lobules. A large amount of coarse brown pigment in the liver-cord cells gave a strongly positive Prussian blue reaction for iron and a negative Hotchkiss reaction for lipofuchsin (Fig. 11). This pigment was minimal in Kupffer cells and absent in the bile duct epithelium, although small deposits were seen in the connective tissue of the portal triads. The lobules appeared fairly uniform, but the pigmentation, atrophy, and hyperemia were slightly less severe in the immediate periportal areas than elsewhere. The gall bladder mucosa contained accumulations of foamy cells, which comprised the tiny yellow nodules seen grossly.

*Urinary System.*—The left kidney weighed 280 gm. and the right 268 gm. The kidneys were plump, firm, dark-reddish organs. The thin capsules stripped easily, leaving smooth surfaces, devoid even of fetal markings. The

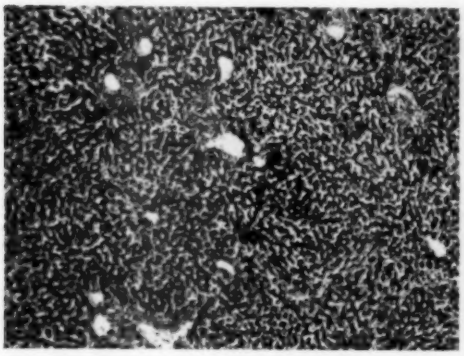


Fig. 10.—Liver. Photomicrograph showing the uniform small lobules with atrophic, heavily pigmented cords; celloidin embedding; hematoxylin-eosin stain; reduced ⅓ from mag. × 57.

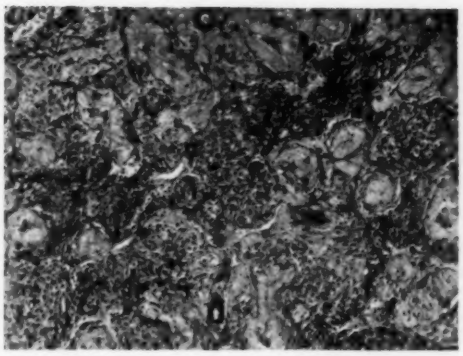


Fig. 12.—Testis. Photomicrograph showing high-grade atrophy and sclerosis of seminiferous tubules and conspicuous interstitial cells of Leydig; celloidin embedding; hematoxylin and eosin; reduced ⅓ from mag. × 95.

cut surfaces bulged to reveal a reddish-brown cortex and a dark reddish-black medulla but no other conspicuous markings. The cortex measured 16 mm., the medulla 28 mm. The normal-appearing, simple, small, funnel-shaped pelvis, without a calyx system, was attached to the kidney. The ureters appeared normal, as did the urinary bladder. It contained about 100 cc. of thin, slightly turbid, pale fluid, and its mucosa was pale.

Microscopically the kidney showed much degeneration of tubular epithelium, considered to have occurred post mortem, an occasional fibrosed glomerulus, and small areas of tubular atrophy. The stain for fat was essentially negative. The bladder appeared normal.

*Generative Organs.*—Two pectoral nipples arose from flat skin without any mound. The penis was small, black, and flabby, with no foreskin and an inconspicuous bulbous glans. The small, nonpendulous, flat scrotum contained soft testes, which weighed 23 and 19 gm. with their epididymides. They resembled large human testes. The cut surfaces were uniform pale tan, and no seminiferous tubules could be teased out.

Microscopically the testes showed a virtually complete atrophy and sclerosis of the seminiferous tubules and a great relative, if not actual, increase in the number of interstitial cells of Leydig in all areas; an unquestionable increase, represented by large solid sheets, was present in many places (Fig. 12).

Fig. 11.—Photomicrograph showing positive reaction for iron brown pigment. It is located chiefly in liver cells. Paraffin embedding; Prussian blue reaction; reduced ⅓ from mag. × 350.

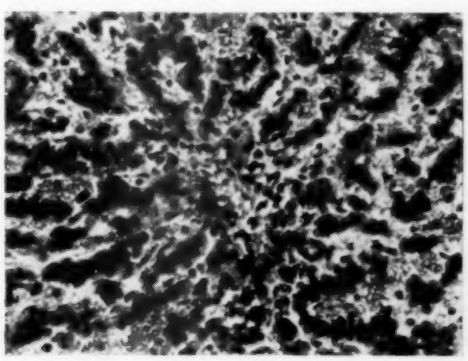
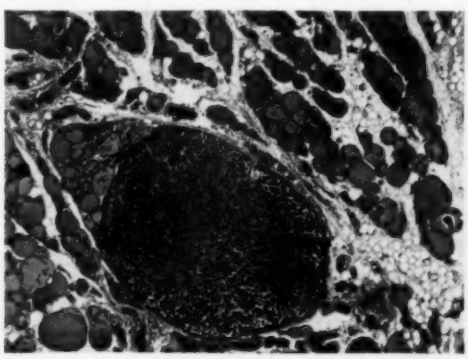


Fig. 13.—Thyroid. Photomicrograph showing abundant fibroadipose stroma and a Hürthle cell adenoma; celloidin embedding; hematoxylin and eosin; reduced ⅓ from mag. × 26.



The remnants of the tubules consisted of rounded, shrunken, solid fibrous or hyaline structures. Some contained occasional foamy spindle or polyhedral cells about small lumens. No spermatozoa or germinative epithelium was found. A few tubules had small lumens, surrounded by variably eosinophilic, foamy, glassy, or fibrillar material, which in some places formed bodies of irregular shape and elsewhere showed as solid rings. An occasional remnant of a Sertoli cell and a few macrophages were present in a small percentage of the tubules. The interstitial cells formed diffuse infiltrates and confluent sheets in which there was an increase in fibrous connective tissue in some places, according to the Mallory-azocarmine G reaction. The interstitial cells were usually polyhedral. Their outlines were distinct in some places and indistinct elsewhere. In size they varied from medium to large. The cytoplasm of the larger cells was paler and sometimes slightly foamy. From the appearance of the other sex organs it is probable that at least some of these interstitial cells were functional. There was no testicular inflammatory process and no great interstitial focal fibrous scarring.

The prostate was small, soft, pale, and symmetrical, measuring 3.2 by 2.6 by 1.0 cm. It was pale tan. Microscopically it showed large glands in a fibromuscular stroma, with no recognizable antemortem change in either. A few small corpora amylacea were present.

The seminal vesicles resembled the human in size and convolutions. They contained a thin, clear, stringy fluid. Microscopically they appeared relatively normal except that they showed no spermatozoa.

*Spleen, Hematopoietic, and Lymphatic Systems.*—The spleen was a relatively plump, tense organ, weighing 372 gm. The capsule was thin and smooth except at the lower pole, where a fibrous adhesion united it to the adjacent diaphragm. The dark purplish-red cut surface was soft and diffluent, and the usual trabecular markings were not seen. Microscopic sections revealed a relatively blood-rich but normal-appearing spleen except for numerous large, brown pigment-containing macrophages located in Malpighian bodies, sinusoids, and cords and found singly or in clusters. The Malpighian bodies were small and probably atrophic.

The lymph nodes were everywhere small, but fairly conspicuous nevertheless, because of their reddish-brown color. The peribronchial nodes showed a slight anthracosis and no tuberculosis. Elsewhere the nodes were pale reddish, which contrasted sharply with the yellowish subcutaneous or whitish internal adipose tissue in which most of them were located. The nodes tended to be about the same size as human nodes in the corresponding locations. The outstanding exceptions were the submaxillary nodes, which appeared large, if not actually enlarged. Microscopically the lymphoid component of all lymph nodes examined appeared atrophic. The tracheobronchial nodes exhibited a small amount of anthracosis, and three cervical nodes showed a definite increase in and slight distortion by fibrous tissue. Several nodes were infiltrated by adipose tissue.

The bone marrow of a lumbar vertebral body was composed of a mixture of fat and hematopoietic tissue, which appeared to have a relatively normal distribution of cell types.

*Endocrine System.*—The pituitary grossly was not remarkable. It was studied in detail by Drs. A. T. and T. B. Rasmussen, who have published a separate paper on this gland.[10]

The thyroid weighed 13.5 gm. It was long, slender, soft, symmetrical, and without gross nodules. It was pale reddish-brown but infiltrated with adipose tissue. Microscopic sections revealed small uniform acini with low epithelium, separated by much edematous fibrous tissue, which was infiltrated by fat (Fig. 13). One encapsulated, 2.5 mm.-sized nodule was composed of large eosinophilic, polyhedral cells, which, probably because of postmortem autolysis, were noncohesive.

The parathyroids were sought but not found, and it was concluded that they were not enlarged.

The thymus was embedded in anterior mediastinal fat, from which it was easily dis-

sected. It weighed 14.8 gm. It was flat, bilobed, and partly replaced by adipose tissue. On microscopic sections, both the lymphoid and the reticular areas were seen, but in small quantity. Hassall's bodies were small and inconspicuous, and they showed various stages of atrophy and degeneration. It is estimated that three-fourths of what grossly appeared to be thymus was adipose tissue.

The adrenal glands weighed 9.3 and 8.5 gm. They were flat and extensively convoluted, as in man. They exhibited an irregular narrow yellow outer cortical layer, a wider brown inner cortical zone, and a narrow pale gray medulla. Microscopically they showed an atrophic, inconspicuous glomerular zone, a slightly irregularly corded and unevenly lipid-poor zona fasciculata, and a narrow atrophic zona reticularis. The medulla appeared normal. The capsular vessels were not sclerotic.

The pancreas weighed 105.5 gm. It was greatly elongated (31.5 cm.) and dark reddish-brown. It showed large lobular markings. It was soft and flabby. Microscopic sections showed severe postmortem degeneration of what appeared to be essentially human pancreas. The islets appeared numerous, large, and conspicuous. A small proportion of the islets were encapsulated by a definite fibrous capsule.

The testes have been described with the generative organs.

*Muscular System.*—The muscles were firm and dark reddish-brown, producing a striking contrast with the abundant yellow fat. The muscles were especially well developed around the shoulder and in the arms. The left leg was smaller in the thigh and calf than was the right leg. In the left lower leg the atrophic muscles were pale and showed an increase in fibrous tissue. Microscopic sections of the left gastrocnemius muscle and other muscles in the left lower extremity revealed a severe atrophy of muscle fibers, accompanied by some reduction in both cross and longitudinal striations, a relative increase in connective tissue, and a little adipose tissue infiltration (Fig. 14). There was no evident inflammation. In contrast, sections prepared from muscles of the right arm and the trunk appeared relatively normal, showing only a slight increase in sheath cell nuclei (Fig. 15).

*Skeleton.*—The bones wherever examined were hard, and the marrow in regions normally red was a medium shade of red. No obvious skeletal abnormalities were seen. The joints also were not remarkable. The left hip and knee joints had pale, smooth synovial linings. Microscopic sections through the capsule of the left knee revealed normal synovial and other structures. A section of the semilunar cartilage was also interpreted as normal.

*Nervous System.*—Gross Study: The calvaria and base of the skull were normal, as was the dura. The sagittal and transverse sinuses were normally patent. The brain weighed 605 gm. The convolutional pattern was normal, and there was no evidence of atrophy or softening. The circle of Willis was symmetrical and of the classic human type. The vessels of the circle of Willis and its principal branches were soft and patent and showed no atheromatous changes.

Serial sections through the right cerebral hemisphere at 1 cm. intervals were normal. The ventricles were normal in size, were not displaced or deformed, and showed normally smooth and glistening ependymal surfaces.

Serial sections through the right cerebellar hemisphere and through the brain stem at 5 mm. intervals were also entirely normal.

The left cerebral and cerebellar hemispheres were preserved for anatomical study.

The spinal cord measured 44 cm. in length and was normal grossly in its external appearance and on sectioning at 1 cm. intervals.

Specimens were taken from both brachial plexuses, both sciatic nerves, the left ulnar, radial, and median nerves, the right sympathetic trunk, the right Gasserian ganglion, and the right axillary artery. These were all grossly normal.

Microscopic Study: Sections were stained with a variety of methods, including the hematoxylin-phloxine, hematoxylin-Van Gieson, Nissl, phosphotungstic acid hematoxylin,

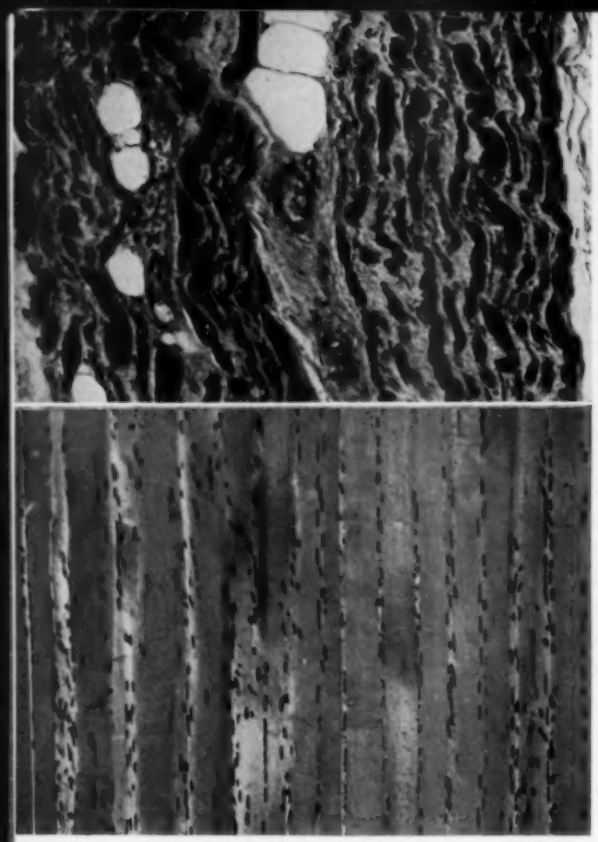


Fig. 14.—Left gastrocnemius muscle. Photomicrograph showing severe atrophy; celloidin embedding; hematoxylin and eosin; reduced 2/7 from mag. × 125.

Fig. 15.—Skeletal muscle. Photomicrograph showing relatively normal skeletal muscle from the right side. Prepared by identical technical conditions as in Figure 14. There is a slight increase in sheath cell nuclei. Reduced 2/7 from mag. × 125.

Pal-Weigert, Mallory connective tissue, and Holmes axis cylinder stains.

Cerebral cortex. Sections studied from the right frontal pole and right parietal and right occipital regions showed a moderate degree of tissue shrinkage as a fixation artifact but no abnormalities in pattern of neuronal or supporting elements. Many of the larger ganglion cells contained collections of lipochrome pigment, such as is commonly seen in human autopsy material from older age groups. Aside from this the neurons were normal. The glial nuclei were normal except for evidence of a moderate degree of acute swelling of the oligodendrocytes, also similar to that seen in human autopsy material. Many of the arterioles and smaller arteries showed moderate thickening of their walls, but the vessels of the parenchyma and leptomeninges were otherwise normal.

Basal ganglia. This section showed rather less thickening of the walls of the smaller arteries than was seen in the previous sections. The neuronal and supporting elements were as described above.

Cerebellum. The Purkinje cells, granular layer, molecular layer, and subjacent white matter were normal except for a moderate degree of acute swelling of the oligodendrocytes.

Pons and medulla. Some of the larger neurons of the tegmental nuclei showed chronic degenerative changes, such as peripheral displacement of Nissl substance, swollen cell bodies, or pyknotic and shrunken cell bodies, but the majority of neuronal elements were normal. The ganglion cells of the olivary nuclei contained considerable lipochrome pigment but were otherwise normal. Some of the arterioles and small arteries showed moderate thickening and hyalinization of the vessel walls. The myelin pattern was normal.

Spinal cord. Sections were studied from the upper and middle cervical; upper, middle, and lower thoracic; lumbar, and sacral regions. There was moderate demyelination in the medial portions of the dorsal columns—the portions related to the afferent inflow from the lumbar and lower thoracic regions. There was less marked but definite demyelination in the midportions of both anterolateral white columns in the thoracic and cervical sections (Fig. 16).

At all levels most of the large neurons of the ventral gray columns showed marked chronic degenerative changes, consisting of diffuse chromatolysis and bulbous swelling of the cell bodies, with eccentric displacement and deformation of the nucleus (Fig. 17). Almost no normal somatomotor neurons were seen. A few diffusely stained or ragged cell ghosts were present, and there was an occasional shrunken pyknotic nerve cell. The small neurons were much more normal, but some of these also showed similar degenerative changes. There was no marked reduction in numbers of ventral horn cells.

Fig. 16.—Upper thoracic spinal cord. Photomicrograph showing moderate demyelination in the medial halves of the dorsal white columns and slight demyelination in the anterolateral white columns; Pal-Weigert myelin stain; reduced ½ from mag. × 18.

Dorsal root ganglia. Six dorsal root ganglia from various regions of the cord were studied. Widespread degenerative changes were present in the neurons of all six ganglia (Fig. 18). Only an occasional normal cell was seen. The earliest degenerative change consisted of pyknosis and shrinkage of the nucleus with diffuse chromatolysis. The next stage consisted of disappearance of the nucleus, shrinkage, vacuolation, and pale staining of the cytoplasm, with beginning hyperplasia of capsular cells. A few cells in this stage of degeneration showed accumulations of lipochrome pigment. Some of the neurons had completely disappeared and were replaced by solid circular nests of capsular cells.

Gasserian ganglion. This structure showed the same type of degenerative changes in the

Fig. 17.—Spinal cord. Chronic degenerative changes in anterior horn cells of lumbar region; Nissl stain; reduced ⅓ from mag. × 250.

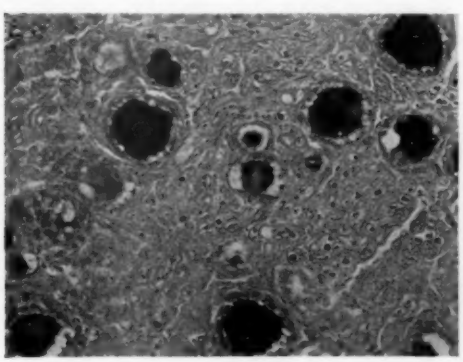
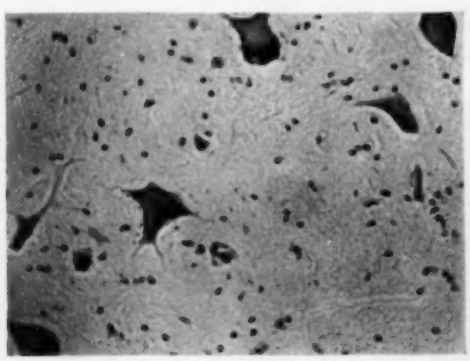


Fig. 18.—Dorsal root ganglion showing severe chronic degenerative changes in neurons; Nissl stain; reduced ⅓ from mag. × 165.



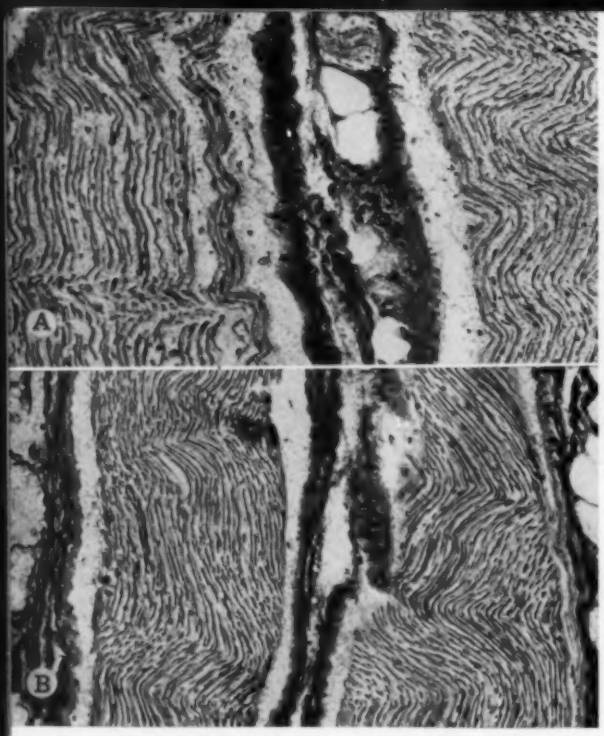
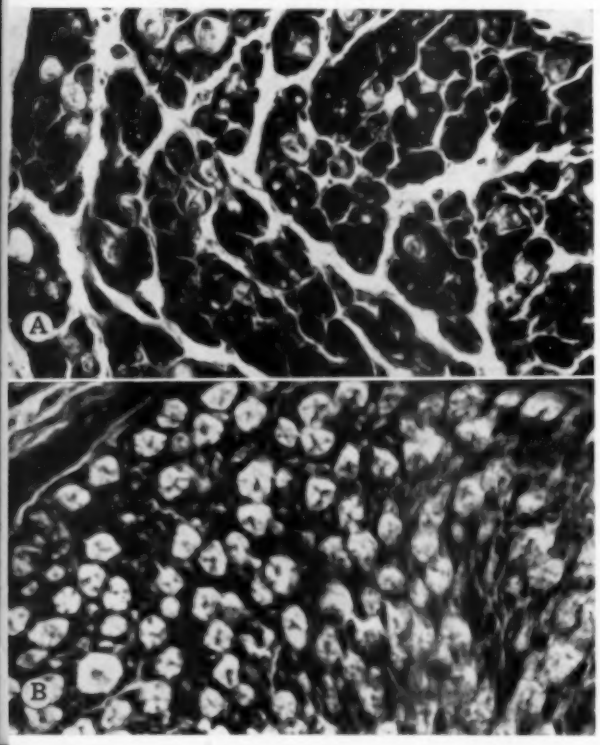


Fig. 19*A* and *B*.—Nerves. Longitudinal sections of the right sciatic (*A*) and the left ulnar (*B*) nerve. Note paucity of axis cylinders in *A* as compared with *B*. Holmes strain; reduced ⅓ from mag. × 125.

Fig. 20*A* and *B*.—Nerves. Cross section of the right sciatic (*A*) and the left ulnar (*B*) nerve. Note the marked fibrosis of neural tubes in *A*. Mallory's connective tissue stain; reduced ⅓ from mag. × 625.



neurons as seen in the dorsal root ganglia, but the proportion of normal cells was considerably higher.

Sympathetic ganglia and trunk. Most of the neurons were normal, but there were occasional degenerating neurons, similar to those described in the previous two paragraphs. The nerve fibers in the sympathetic trunk appeared normal.

Peripheral nerves. In the sciatic nerves there was marked loss of both myelin tubes and axis cylinders, especially the large ones, with compensatory thickening of the endoneurium, giving a picture of marked fibrosis of the nerve bundles (Figs. 19, 20, and 21). One or two actively degenerating myelin tubes could be seen in each nerve fascicle. The nerves from the arms were more normal, but in each nerve fascicle 1 to 10 empty dilated tubes were present.

Summary: The neurological autopsy findings revealed a widespread chronic degenerative process involving the neurons of all levels of the spinal cord and the dorsal root ganglia, with secondary demyelination and disappearance of axis cylinders in the peripheral nerves and with demyelination in the ascending tracts of the spinal cord. Only a few of the neurons of the medulla and pons were similarly affected, and none of the cells of the upper brain stem, cerebellum, or cerebrum showed any such atrophic or degenerative changes. The histological characteristics of the degenerating neurons ruled out the possibility that these were retrograde changes secondary to primary lesions of the peripheral nerves. There were no changes present to suggest a postinflammatory state, and it seemed clear that the nervous system lesions represented a primary degenerative process.

*Diagnosis.*—The final pathological anatomical diagnoses were as follows:

1. Diffuse chronic degenerative process involving the neurons of the spinal cord and the dorsal root ganglia, with secondary demyelination and disappearance of axis cylinders in the peripheral nerves and with demyelination in ascending tracts of the spinal cord.

2. Slight arteriosclerosis of the cerebral hemispheres and spinal cord.

3. Atrophy of the muscles of the extremities, especially the left lower.

4. Eversion and flexion deformity of the left foot, with absence of the distal end of the fifth toe; chronic ulcer of the left heel.

5. Dilatation of the heart, especially the right chambers; interstitial myocardial edema; diffuse fatty change and fragmentation of myocardial fibers; focal and diffuse myocardial fibrosis, and arterial and arteriolar sclerosis of occasional small myocardial vessels.

6. Hemosiderosis and atrophy of the liver and of the spleen.

7. Severe tubular atrophy and fibrosis of the testes.

8. Acute hypostatic pulmonary hyperemia and edema.

9. Cloudy swelling of the kidneys.

10. Petechial hemorrhages in the thymus, stomach, and colon.

11. Multiple focal fibrous peritoneal adhesions.

12. Hürthle cell adenoma of the thyroid.

13. Obesity, including cardiac obesity.

### INTERPRETATION AND COMMENT

The findings indicate that the immediate cause of death was acute congestive heart failure, characterized clinically by dyspnea and morphologically by hypostatic pulmonary hyperemia and edema and passive congestion of the liver, both of short duration according to the microscopical appearances. The etiology of this cardiac failure is best explained in the light of the other necropsy findings and the collateral evidence. It is clear from the clinical history that death came rather suddenly in the course of a subacute illness of about seven months' duration and a chronic lesion of about six years' standing. In retrospect, it is even possible that the "arthritic-like" illness 17 years before death may also have been a part of the same fundamental illness.

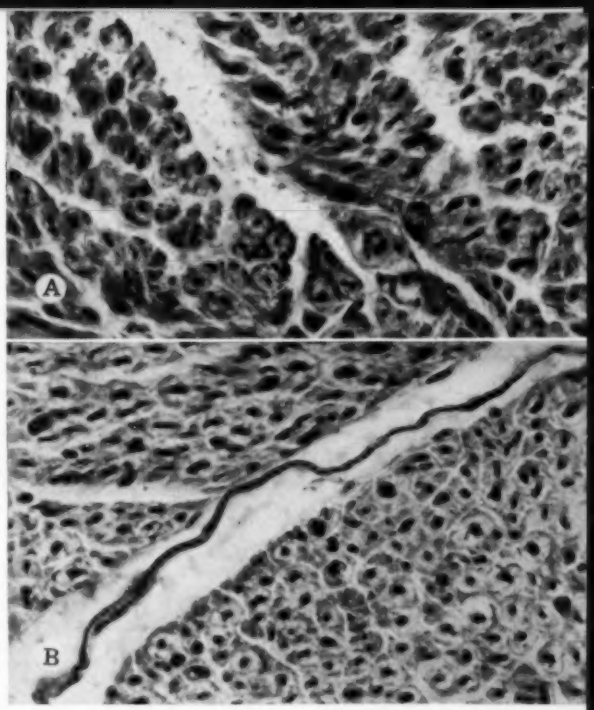



Fig. 21*A* and *B*.—Nerves. Cross section of the right sciatic (*A*) and the left ulnar (*B*) nerve. Note the reduction in number of axis cylinders in *A* as compared with *B*. Holmes stain; reduced ⅓ from mag. × 625.

The principal abnormalities were the neuropathy and related lesions in the muscles and the foot, the cardiopathy and the change in the arteries, the hemosiderosis of the liver and spleen, and the testicular sclerosis. These changes appear primarily degenerative rather than postinflammatory. The most probable interpretation is that they were nutritional in origin. Taken in conjunction with the observations that male gorillas in captivity have not shown spermatogenesis, although the other three anthropoid apes—the orangutan, chimpanzee, and gibbon—have reproduced, and that they have an unexplained restricted natural habitat, the facts may jointly indicate that the gorilla has a special nutritional requirement not possessed by the other primates. Whether the ovaries also become atrophic is not known; we have been unable to find any histological description of an adult gorilla ovary, either wild or captive.

The exact nature of the nutritional defect is uncertain. The general nutrition appeared good. In fact, the animal was obese, and the general musculature was well preserved.

There was no inanition. The selective and localized nature of the lesions points to some special deficiency. It appears that the findings are characteristic not of any one deficiency but, rather, of a group, most likely the vitamins, especially members of the B complex, and possibly vitamin E.

The interpretation is made difficult by the fact that an exact determination of the degree and extent of some of the changes, such as the alteration in size and shape of the heart, is impossible, because of the paucity or absence of available normal standards. Very few observations on gorilla viscera are on record. Furthermore, organ weight to body weight ratios are not entirely satisfactory because of the variations in body fat. Adult gorillas in captivity usually weigh more than those in their natural habitat. Whether some of this added weight represents true healthy body structures or whether it is entirely due to pathological obesity is not known. Bushman had dropped from a maximum of 550 to about 500 lb. and still had abundant, if not excessive, quantities of depot fat. If one can generalize from these observations, some of the other captive specimens probably do also, and the few available ratios of organ to body weight are, therefore, not good indices.

The interpretation of a nutritional disorder is also complicated by the paucity of information on requirements in this species.[11] Day [12] reviewed the literature on nutritional requirements in subhuman primates in 1944 and found less than 100 papers. Most were on rhesus monkeys; a few were on anthropoid apes, and none dealt with the gorilla. The interpretations which follow must be made largely by analogy with other species, including man.

Despite these handicaps, an attempt has been made to get a rough idea of average, if not normal, organ values by compiling the available data, as in the Table. Considering these data, it is probable that the heart of Bushman was slightly oversize, the liver atrophic in addition to being hemosiderotic, and the pancreas and thymus atrophic. The lungs were overweight, because of the terminal congestion, but not oversize. The kidneys were probably slightly increased over normal as a part of the terminal congestive failure. Adrenals in fatal illnesses that are not sudden are usually overweight, despite much late depletion of their lipid.

The predominant lesion, observed clinically and postmortem, was in the nervous system. The atrophy of skeletal muscles and

*Organ Weights and Ratios to Body Weight in Gorillas*

| | Bushman (Chicago) | | Bobby (Berlin) | | M'Bongo (San Diego) | | Lady Congo (Brookfield) | |
|---|---|---|---|---|---|---|---|---|
| Body weight, kg..... | 228.2 | | 241.8 | | 264 | | 147.7 | |
| | Weight, Gm. | Percentage Organ Weight to Body Weight | Weight, Gm. | Percentage Organ Weight to Body Weight | Weight, Gm. | Percentage Organ Weight to Body Weight | Weight, Gm. | Percentage Organ Weight to Body Weight |
| Heart..... | 805.0 * | 0.353 | 560.0 | 0.232 | 1,134.0 * | 0.430 | 341.0 | 0.230 |
| Right lung..... | 1,110.0 * | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Left lung..... | 850.0 * | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Omentum..... | 3,700.0 * | 1.621 | 1,700.0 | 0.703 | ..... | ..... | ..... | ..... |
| Liver..... | 2,200.0 † | 0.964 | ..... | ..... | 5,000.0 * | 1.894 | 1,510.0 | 1.020 |
| Kidneys..... | 548.0 | 0.240 | ..... | ..... | ..... | ..... | 283.0 | 0.198 |
| Spleen..... | 372.0 | 0.163 | ..... | ..... | 680.0 | 0.257 | 150.0 | 0.101 |
| Pancreas..... | 105.5 † | 0.046 | ..... | ..... | ..... | ..... | 147.0 | 0.100 |
| Thyroid..... | 13.5 | 0.006 | 17.0 | 0.007 | ..... | ..... | 12.3 | 0.010 |
| Thymus..... | 14.8 † | 0.006 | ..... | ..... | ..... | ..... | ..... | ..... |
| Adrenals..... | 17.8 * | 0.008 | 15.0 | 0.006 | ..... | ..... | ..... | ..... |
| Testes..... | 42.0 ‡ | 0.018 | 10.5 § | 0.004 | ..... | ..... | ..... | ..... |
| Brain..... | 605.0 | 0.265 | ..... | ..... | 565.0 | 0.214 | ..... | ..... |
| Pituitary..... | 1.59 | ..... | 1.36 | ..... | ..... | ..... | ..... | ..... |

* Regarded as probably pathologically increased over normal.
† Regarded as probably pathologically decreased under normal.
‡ With epididymides.
§ Without epididymides.

the changes on the foot were probably secondary to the neurological defect.

Degenerative changes in the nervous system have been described in deficiencies of the vitamin B group. They have been associated with insufficient thiamine (vitamin $B_1$), riboflavin (vitamin $B_2$ or G), nicotinic acid, pantothenic acid, pyridoxine (vitamin $B_6$), and biotin.§ The lesions have not all been produced in every species, and they have not all been described in man. In some instances, the lesions may have been caused by multiple deficiencies rather than by single factors.

If the ulcer on the heel and the erosion of the fifth toe are accepted as trophic or consequent to sensory or other nerve damage, then the neurological disorder is of long standing—at least six years. If the nerve changes are consequent to vitamin deficiencies, the latter were probably prolonged but marginal. The lesions would, therefore, be somewhat different from those produced acutely by nearly total deprivation.

Degenerative changes in the skeletal muscles have been described in vitamin deficiencies. They may be primary, as in vitamin E deprivation, or secondary to degeneration in the nervous system. The character of the atrophy and its limitation to areas showing severe degeneration in nerves indicate its secondary nature in the present case.

The cardiovascular lesions also are explainable on the basis of deficiencies in the vitamin B group, although not to any one compound alone. Heart changes have been reported in man in beriberi and in biotin deficiency, as well as in potassium deficiency. They have been produced experimentally also in vitamin E (alpha-tocopherol), pyridoxine, and ascorbic acid deficiencies.[13] Except for the vascular lesions, the cardiac changes found in Bushman are compatible with the picture described in beriberi heart, although, like the nervous system lesions, they are not pathognomonic for thiamine deficiency. The slight hypertrophy and dilatation, especially of the right ventricle, the fatty change, and the interstitial edema might be so interpreted. The myocardial fibrosis shown in Figure 8 appears to be a duplicate of that shown by Follis [13] for swine in thiamine deficiency (his Figure 48). The hypostatic acute pulmonary hyperemia and edema indicate a terminal cardiac insufficiency, and the clinical observation of dyspnea falls in line. Except for the absence of an alcoholic history, our case resembles in the heart and nervous system findings those described by Blankenhorn and co-workers [16] in human occidental beriberi heart disease, by Schlesinger and Benchimol,[17] and by Gillanders [18] in Africans, in whom the condition was accompanied by iron deposits in the liver and spleen, as in the present case.

Thiamine requirements are proportional to carbohydrate combustion, and a diet consisting chiefly of carbohydrate is said to be an important contributing factor in the development of thiamine deficiency.[14] Bushman's diet consisted largely of carbohydrates, supplemented by milk as the chief source of protein. The myocardial scarring produced by deficient potassium is similar to that produced by insufficient thiamine, but there is no reason to suspect a potassium deficit. Myocardial necrosis and scarring have also been reported in alpha-tocopherol deficiency, but this lesion is accompanied by deposition of ceroid, a feature not found in Bushman.

The myocardial scarring in Bushman resembled that sometimes attributed in man to miniature infarctions. The coronary sclerosis was rather severe in some of the intramyocardial branches, but in no vessel observed did it lead to great reduction in caliber of the artery concerned. The scars are, therefore, probably a sequel to necrosis of some type other than vasculo-occlusive ischemic necrosis.

Arteriosclerosis has recently been produced by pyridoxine (vitamin $B_6$) deficiency in rhesus monkeys by Rinehart and Greenberg.[19] Their illustrations and the lesions here illustrated in the heart and aorta show a remarkable similarity. They appear different from those produced by Mann and associates [20] in Cebus monkeys by a diet rich in

§ References 13 through 15.

cholesterol and deficient in the sulfur amino acids methionine and cystine. The lesions of insufficient pyridoxine were chiefly fibrotic, whereas the latter were rich in lipids.

It is of additional interest, if not importance, to the present case that pyridoxine-deficient dogs have developed cardiac deficiency and died suddenly.

Hemosiderosis is found under many conditions. It may follow hemolysis, many transfusions, and deficiency states. Although fatty livers have been described in inanition and in many vitamin deficiencies (riboflavin, pantothenic acid, pyridoxine, choline), hemosiderosis of the liver and spleen has been reported in experimental avitaminoses only in pyridoxine deficiency with anemia. Its appearance in the present case lends further support to the idea of deficiency in the vitamin B group. The liver pigment was far greater than that found in aging, and there are other reasons for concluding that Bushman was not senile. Gillman and Gillman described liver hemosiderosis in pellagra in Africa [21] and also in many persons without overt pellagra.[22] They divided the liver damage, from early fatty changes to cirrhosis with iron pigment, into four stages. Bushman's liver would fall into their Type 2, in which the iron-containing pigment is found chiefly in liver cells.

Atrophy of the seminiferous tubules of the testes with preservation of the interstitial cells of Leydig has been caused by a number of methods, including severe inanition. A specific type of irreversible atrophy of the seminiferous tubules has been produced in rats by vitamin E (alpha-tocopherol) deficiency by Pappenheimer and Schogoleff.[23] In none of the experiments has the tubular epithelium been accompanied by the extreme sclerosis seen in the present case. Whether this difference is explained by the shorter time in the experiments or whether it indicates that the lesions here described had some other etiology, we cannot say. We are inclined to the latter view, because the diet was rich in green leafy vegetables and cereals and it would be expected to have provided adequate amounts of vitamin E. The degeneration could hardly have been on the basis of inanition. The gorilla may have especially large quantitative requirements for vitamin E or for some other, unknown factor necessary to maintain the integrity of the seminiferous tubules. Alpha-tocopherol deficiency is said not to affect the ovaries. If the structural integrity of gorilla ovaries could be demonstrated in zoos in which both sexes were on the usual diet but the males had developed sclerosis, this observation would speak for a deficiency in some highly selective substance, of which alpha-tocopherol is a prime example.

That testis lesions of this sclerotic type are not congenital but acquired is indicated by their occurrence in men who were known to have previously been fertile. Nelson and Heller [24] and Sniffen and co-workers [25] have described human testis sclerosis from unknown causes similar to that found in this gorilla. The changes in the testes of this gorilla appear severer and older than those in the liver; so they are probably not consequent to the liver damage.

Interpretation of the present findings is aided by two autopsy reports and scattered comments on other gorillas found in the literature. The poliomyelitis-like disease mentioned by Hooton [4] in two gorillas (Massa and Janet Penserosa) and by Freeman (Lady Congo) ‖ is now seen to be something else. The precipitation of the disease in Massa by an attack of pneumonia and his gradual recovery on a liver and meat diet speak for a nutritional etiology. Freeman also observed some improvement in Lady Congo with B vitamins and liver. The autopsy report by Kennard and Willner,[3] presumably on Janet Penserosa, and the one by Schultz [5] state that this gorilla had had paralysis of the lower extremities for two years and that the extremities were markedly atrophic, although the rest of the animal was normally developed and in excellent nutrition.

The atrophy of seminiferous tubules illustrated in Bushman (Fig. 12) is a duplicate of that reported by Koch [7] for Bobby in his

‖ Freeman, S.: Personal communication to the authors.

Figures 11 and 12. He considered this testicular change as secondary to a cyst in the pituitary. This explanation loses ground in the light of the present case, in which no such cyst was present.[10] McKenney and associates [8] made smears from the small testes in M'Bongo, the 15-year-old San Diego mountain gorilla. Finding no sperms, they concluded that he was still sexually immature, although Schultz [26] had stated that the three great apes reach adulthood at practically the same age: the permanent dentition is complete in the 11th year, and general growth ceases between 10 and 12 years of age. In view of these comments and of the testicular changes in Bushman and Bobby, it is probable that M'Bongo also had similar atrophy, and the conclusion that he was immature may have been premature. According to Hediger,[6] the gorilla Alfred in the Bristol Zoo (England) had testes weighing only 15 gm. although he weighed 490 lb.

There is every indication that the testicular lesions represent an atrophy and not a hypoplasia. The wavy character of the sclerosis showed that collapse had occurred in what had once been larger tubules. The relative absence of interstitial fibrosis speaks against a previous orchitis. The testes, as well as the penis, of captive and wild gorillas always appear small for the size of the animal.¶ Hooton makes the statement that the external genitalia of the great apes undergo atrophy as they mature, unlike those in man. One is inclined to interpret such a change as pathological rather than as normal.

The life span of gorillas is not known. In their natural habitat it cannot be accurately determined, and in captivity most specimens have died at a young age.[2] Schultz,[5] from morphological observations, arrived at a figure of about 10 years for adulthood. Janet Penserosa was said to have menstruated once for three days at the age of 9 years.[4] Two male specimens showed no sexual activity at the ages of 8 and 8½ years in over 200 hours of observation by Carpenter.[28] At the time of his death, Bushman was the second oldest gorilla in captivity, and it was of interest to determine whether he showed any evidence of aging. None was found. The general condition appeared good, and skin turgor was firm. There was no graying of the hair. The musculature in general was well preserved. Except for the specific changes already mentioned, the cardiovascular and urinary systems showed no retrogressive changes. It is concluded that this gorilla was not senile, but beyond this point no deductions on age are made.

The diet of wild gorillas has been described by Bingham,[29] Gregory and Raven,[30] Hooton,[4] and by others. It is believed to consist chiefly of fruit and vegetation. The source of proteins is not known and it is not definitely known that they are purely herbivorous rather than omnivorous. Bingham # saw rotten logs that had been torn apart, possibly during a search for insects and worms. In zoos gorillas are usually considered herbivorous, but some specimens have been taught to eat meat. When they were young, Gargantua and Massa were observed to pursue caterpillars and small animals; so they were fed meat.[4] Toto also was taught to eat meat.[31] In view of the pathological findings in the present case, this question of diet needs to be reconsidered.

Although it is known that captive anthropoid apes often present problems in feeding, this report appears to be the first description of the effects of malnutrition and of a vitamin deficiency.

The fibrous peritoneal adhesions in the anterior part of the abdomen appeared to be incidental to the main findings, and their etiology is unknown. It is of special interest that the gorilla described by Koch [7] had similar adhesions. In man they are rarely seen in this location and to this extent, except as a postoperative finding. The quadrupedal posture of the gorilla may explain their location but not their etiology.

In May, 1954, a young gorilla at the Zoo became ill, with weakness in both forearms and one leg. He had accidentally been ex-

¶ References 26 and 27.

# Bingham, H. C.: Personal communication.

posed to lead paint. Large amounts of lead were found in his urine, and the diagnosis of lead neuritis was made. A detailed report of this case will be made later. We had considered the diagnosis of lead poisoning in Bushman but dismissed it from prime probability, realizing that the possibility cannot be eliminated. The cardiac and vascular changes are unlike those described in lead poisoning.[32] The changes produced by lead in the liver are hepatocellular degenerations rather than pigmentation,[32] and many abnormalities have been described in other tissues which were not found in the present case. The nervous system lesions may, apparently, be similar in the two conditions.* Testicular damage in lead poisoning has been both affirmed and denied.† This is obviously a subject that needs further investigation.

## SUMMARY

The clinical and postmortem findings in a lowland gorilla aged 22 years are presented and discussed. The immediate cause of death was an acute passive congestion, chiefly of the lungs, of the type associated with cardiac insufficiency. The chief pathological findings were degenerative in nature and selective in location. They were found in the nervous system and the related muscles and one foot, in the heart and arteries, in the liver and spleen, and in the testes. In the absence of generalized malnutrition and inanition, and in the presence of a clinical history of long duration with exacerbations, and in view of the peculiar focal nature of the lesions, the interpretation is made that the findings were most probably nutritional in etiology. They are compatible with the idea of a hypovitaminosis of several members of the B group and, possibly, also vitamin E and other unknown factors. The changes in the nervous system and heart are suggestive of those in chronic dry beriberi; those in the small arteries, of pyridoxine deficiency; those in the liver and spleen, of pellagra and/or pyridoxine deficiency, and those in the testes, of avitaminosis E or other factors. The possibility that these changes represent a lead poisoning cannot be excluded.

From the facts that a paralytic disease has occurred a number of times in captive gorillas; that the other three species of anthropoid apes have sexually matured and reproduced on zoo diets but gorillas have not; that several captive gorillas have had testicular atrophy and sclerosis, and that the gorilla in nature has had a peculiar and restricted range, it is deduced that the gorilla may have some special and unique quantitative or qualitative nutritional requirement which must be met if this species is to maintain health and reproduce. The danger of extinction of this species makes further clinical and pathological studies imperative.

Mr. R. Marlin Perkins, Director of the Lincoln Park Zoological Gardens; Drs. John Edgcomb and Nathan R. Brewer, and members of the staff of the Chicago Museum of Natural History assisted in this study. Dr. Smith Freeman and the Chicago Zoological Park, Inc., Brookfield, provided data on Lady Congo.

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# EXPERIMENTAL STUDIES ON HISTOGENESIS OF BLOOD-BORNE METASTASES

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With the Assistance of Joseph Baum, B.S.

THE FATE of tumor cell emboli carried by the blood stream to distant organs has been investigated experimentally by various workers.* In all these investigations tumor cell suspensions were injected into the circulation of the animal, usually into the vein of the tail or into the left ventricle of the heart. It was shown that only a small percentage of the injected tumor cells survived the great majority of the embolic cells failing to establish themselves and form tumor.† This observation is in agreement with the finding in autopsy material of regressing tumor cell emboli in the arterioles of the lungs of tumor-bearing patients.‡ On the basis of both autopsy and experimental evidence, it was concluded that "tumor embolism is not metastasis,"[17] a principle which was first enunciated by Goldmann in 1897.[18] While there is sufficient agreement on this point, other aspects of the fate and evolution of blood-borne tumor cell emboli are still matters for discussion. One of the controversial points is whether blood-borne metastases arise from viable tumor emboli which have been arrested in the arterioles or from those lodging in the capillaries. Willis[17] attributes equal importance to both sources, while Iwasaki[5] and Takahashi[8] are in favor of the arteriolar source. On the other hand, Ewing[19] does not even mention the possibility of metastases arising from arterioles, and Walther,[20] in his exhaustive investigation of autopsy material, stresses the importance of the capillary bed as a filter where the tumor cell emboli are arrested. Coman and associates,[1] using the Brown-Pearce rabbit carcinoma, found that at least the majority of metastatic growths arise from tumor cells lodging in the capillaries; Warren and Gates[9] also state that "if the tumor lodges in an arteriole, direct penetration is infrequent."

Another controversial point is the question of the capacity of tumor cells to establish growth in the lumen of the blood vessels at the site of arrest; proliferating tumor cells in the lumen of pulmonary arterioles were described by Takahashi,[8] whereas Warren and Gates[9] found that tumor cells "apparently are unable to establish permanent growth in the blood stream."

A third controversial point concerns the number of cells constituting a viable tumor embolus. Coman, de Long, and McCutcheon[1] found that single tumor cells or tiny clusters were more successful in producing tumors than were large clumps, a finding which is at variance with some reports based upon autopsy material§ and with the experimental results of Watanabe[10] and of Weil.[11] Despite the numerous investigations on metastatic spread, there is a surprising lack of information concerning the histogenesis in the earlier stages of blood-borne metastases in man, while the experimental studies mentioned above were based on the method of injecting a tumor cell suspension into the circulation of the animal. It is evident, however, that intravenous injection of tumor

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From the Division of Oncology, the Chicago Medical School.

* References 1 through 12.

† References 5, 8, 9, 12, and 13.

‡ References 5 and 14 through 17.

§ References 16 and 17.

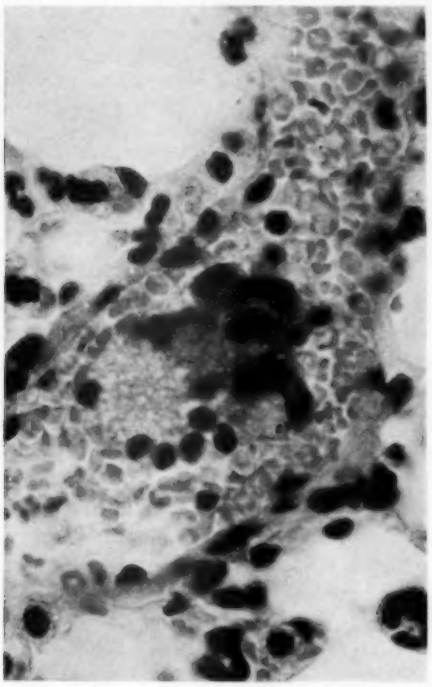


Fig. 1.—Tumor cell embolus lying free in the lumen of a pulmonary arteriole; hematoxylin and eosin; × 600.

cells is not strictly comparable to the mechanism of spontaneous blood-borne metastases in either man or animal.‖

An accurate knowledge of the earlier stages of metastatic growth seems essential to ensure a better understanding of the peculiarities of metastatic spread and a correct evaluation of experimental results.

The present study was undertaken in order to observe the pathogenesis of spontaneous blood-borne metastases in laboratory animals. The material consisted of the lungs of C57 black mice bearing a transplantable tumor, designated as T150. The tumor, obtained through the courtesy of Dr. D. R. Coman, of the Department of Pathology, University of Pennsylvania School of Medicine, originated in the bladder epithelium of a C57 black mouse in the laboratory of Dr. Margaret Lewis. This tumor, when transplanted into the subcutaneous tissue of C57 black mice, metastasizes spontaneously to the lungs and, at least in our experience, not to other organs. The first microscopic metastases can be detected as early as the 14th day after the implant; grossly visible metastases appear 16 days after the implant, and eventually all mice show metastatic growths in the lungs. After the 25th day the metastases are so numerous that it is not uncommon to count from 30 to 50 grossly visible pulmonary growths per mouse. Such metastatic spread provides an excellent source for investigating the histogenesis of pulmonary metastases, from the early stages to the largest growths.

‖ References 17 and 21. Petersen, W., and Colmers, F., cited by Iwasaki.[5]

## METHOD

C57 black mice, male and female, from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, were used. The animals were housed in plastic cages and fed Rockland mouse diet, with water ad libitum. All the animals received transplants of 0.3 cc. of a dense suspension in saline of tumor T150 in the subcutaneous tissue of the right lumbar region, using a 22-gauge needle.

Fig. 2.—Single tumor cell floating free in the lumen of a capillary; hematoxylin and eosin; × 600.

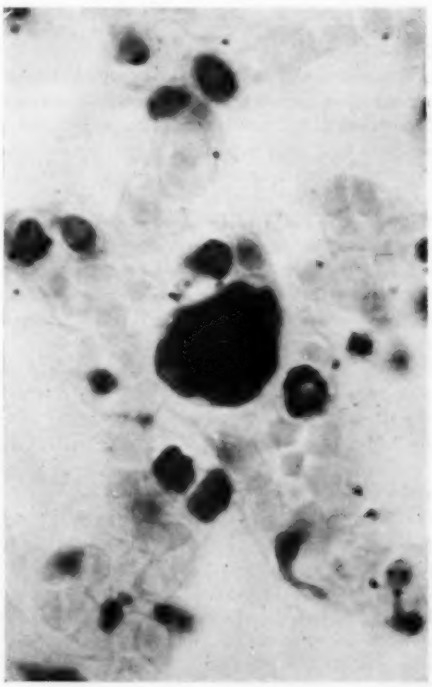


Fig. 3.—Tumor cell embolus adhering to the wall of a pulmonary artery; Weigert and Van Gieson stains; × 600.

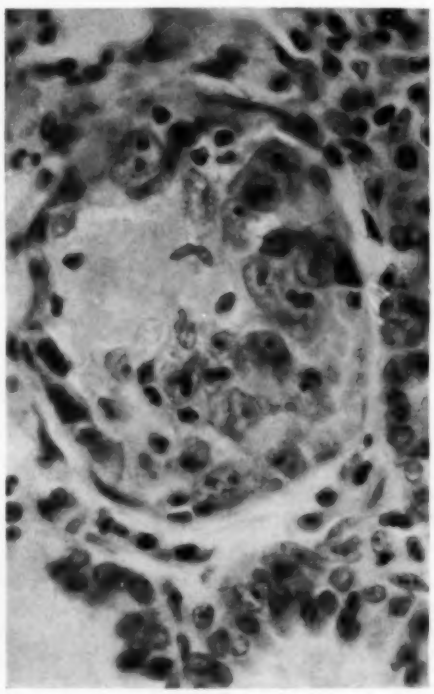
The great majority of the animals included in this experiment were mice used for the routine transplantation of the tumor or groups of mice that served as untreated controls in other experiments. This brought the number of mice included in this study to 130, with a total of 2,140 metastases, an average of 16.5 metastases per mouse, all of which were studied histologically on serial sections. Some animals were killed early, between the 10th and the 20th day after the implant; others were allowed to die. The animals killed between the 16th and the 20th day offered the best source for histological studies, yielding a good number of metastases that had not yet grown to full development. In later stages, the metastatic growths were so large and necrosis was so extensive as to destroy completely any structure, making it impossible to recognize the point of origin.

Autopsy was performed on all animals; the primary growth and the internal organs were examined. The heart and both lungs were removed en bloc and fixed in formalin, and after fixation the grossly visible metastases were counted with the aid of a dissecting microscope. After the count, during which any manipulation of the lungs was avoided, the lobes were separated from each other and embedded in paraffin, and single blocks were made of each lobe. Each paraffin block was cut in serial sections (5 to 6 μ), and the sections were stained with hematoxylin and eosin and the Weigert elastin method combined with Van Gieson's stain. Some sections were stained with a variant of the Bielschowsky-Maresch method for reticulum fibers or with the Highman acid thiazin dye method for mast cells. The number of metastases was again counted at the microscope, and the final figures are those obtained through the microscope.

## RESULTS

Tumor T150 is an anaplastic carcinoma, consisting of very large cells with vesicular nuclei containing from one to three nucleoli. Invasion of blood vessels of the primary growth by tumor cells was a constant finding. From a histological standpoint, the tumor has the advantage that the cells are easily recognizable and distinguishable from the connective tissue cells of the lung and from the bronchial epithelium. The findings in the lungs will be described under separate headings, following what appears to be a logical chronological pattern in the establishment of a metastatic growth.

Fig. 4.—Mural tumor embolus in a pulmonary arteriole; hematoxylin and eosin; × 600.



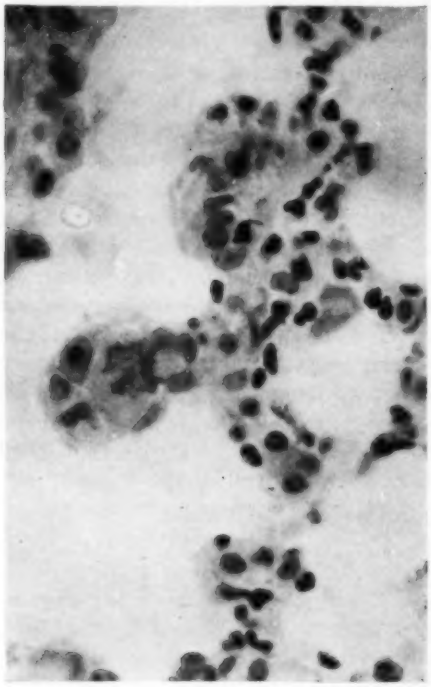


Fig. 5.—Tumor cell in mitotic division in a capillary of the lung; hematoxylin and eosin; × 600.

1. *Carriage of Tumor Emboli by the Blood Stream.*—In some instances isolated tumor emboli, lying free in arteries of the lungs, were found. The largest tumor embolus in our material consisted of seven tumor cells (Fig. 1) lying in the lumen of a terminal arteriole. In most cases the emboli consisted of two to four tumor cells, and in a few instances free single tumor cells were found in the lumen of arterioles.

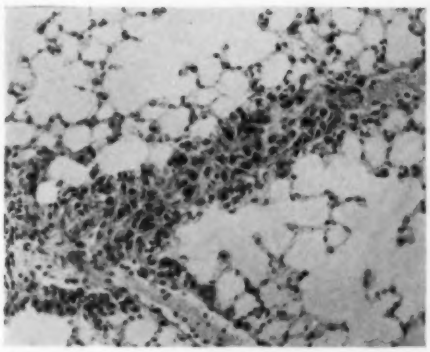


Fig. 7.—Tumor embolus in a pulmonary arteriole with beginning spread beyond the arteriolar wall; hematoxylin and eosin; reduced ½ from mag. × 600.

Much more frequent was the finding of single tumor cells floating free in the lumen of capillaries and far away from any microscopic metastatic implant (Fig. 2). The finding of single intact tumor cells in the lung capillaries of tumor-bearing mice has been reported recently by Watanabe,[10] though, in his material, grossly visible metastases were absent.

2. *Site of Arrest of Tumor Emboli.*—The smallest, and possibly the earliest, fixed tumor emboli were found adhering to the endothelial wall of arteries and arterioles. In only one instance, three tumor cells were found adhering to the endothelial wall of a large artery (Fig. 3). On several occasions

Fig. 6.—Tumor embolus and subsequent thrombus in a large pulmonary artery; hematoxylin and eosin; reduced ½ from mag. × 160.

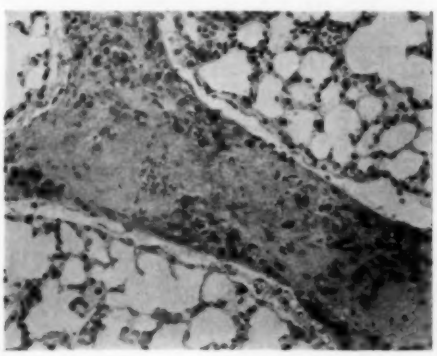
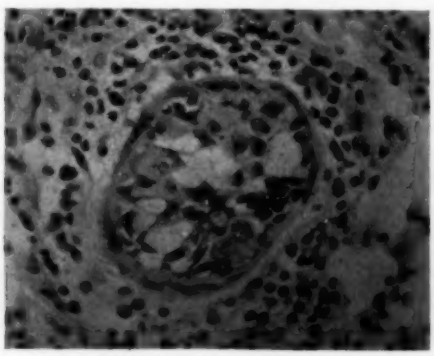


Fig. 8.—Tumor cell embolus and subsequent thrombus in a pulmonary arteriole; hematoxylin and eosin; reduced ½ from mag. × 600.



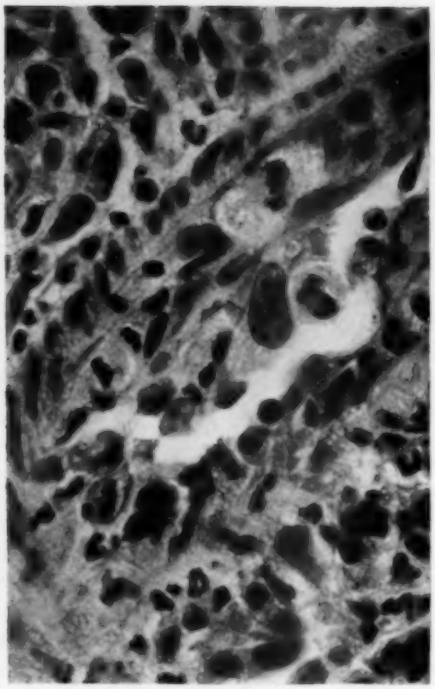


Fig. 9.—Tumor cell embolus in a pulmonary arteriole. Note the mitotic figures; hematoxylin and eosin; × 600.

parietal or mural thrombi of tumor cells were observed in medium-sized arteries or in arterioles (Fig. 4). Early emboli in the capillaries were also observed.

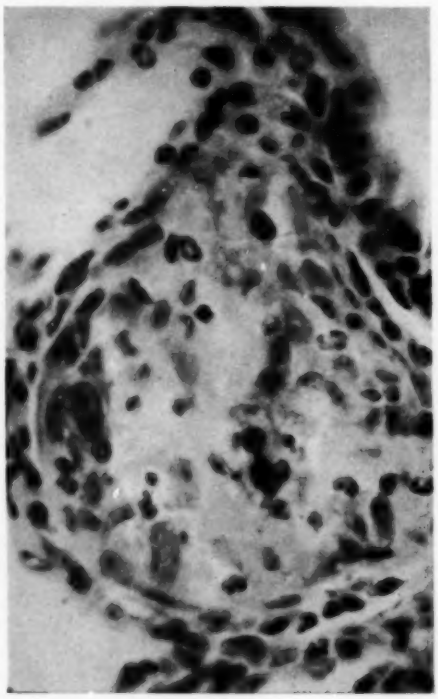
3. *Intravascular Growth of Viable Tumor Emboli.*—From these early tumor emboli arrested in the arteriolar and capillary system of the lungs, all stages of transition were found, from obliterating thrombi to well-established extravascular growths. In the capillaries, the tumor cells proliferated along the lumen; signs of mitotic activity in the lumen of the capillaries were often found (Fig. 5).

In the arterioles, after the cells had adhered to the endothelial wall, there was formation of a thrombus. In some instances these thrombi were much larger than the tumor emboli alone and sometimes so conspicuous as to bring forth an anatomical obliteration of the lumen, though the tumor cells were by no means so numerous as to produce the occlusion by themselves. A striking case is shown in Figure 6.

In other instances, it was obvious that after the formation of the thrombus the tumor cells had proliferated, invading and almost completely replacing the clot, so that at a certain stage the lumen was obliterated by tumor cells, though remnants of fibrinous material could still be found (Figs. 7 and 8). Eventually, the tumor cells proliferated downward to the terminal arterioles and capillaries. On serial sections, it was found that the length of these proliferating and obliterating tumor emboli varied from a few microns to 60 $\mu$. Mitotic figures were found in arterioles (Fig. 9), as well as in capillaries. This observation, to our knowledge, has not been previously reported.

4. *Fate of Tumor Emboli.*—Not all the tumor emboli were viable. In 14 instances, all of which occurred in mice that had been killed 14 days after the implant, regressing tumor emboli were found in the lumen of

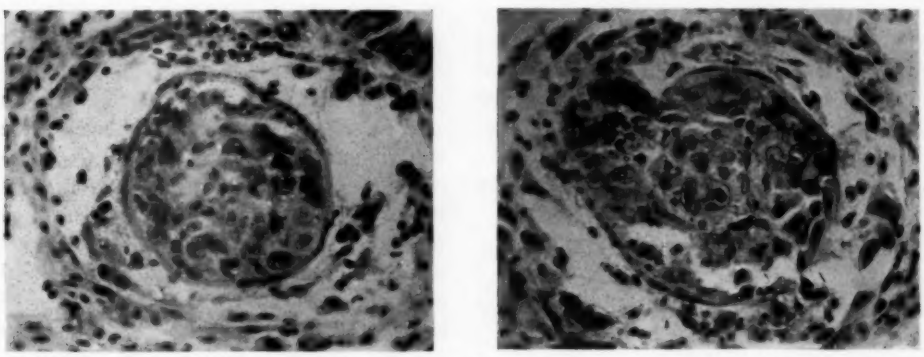
Fig. 10.—Degenerating tumor embolus in a pulmonary arteriole; hematoxylin and eosin; × 600.



pulmonary arterioles (Fig. 10). Such a finding, which is comparatively rare with tumor T150, is much more frequent in the lungs of Swiss mice bearing a subcutaneous methylcholanthrene-induced tumor.¶ Regressing or degenerating growths in an extravascular position were not found in our material.

5. *Passage Through the Endothelial Barrier.*—The viable tumor emboli rapidly spread beyond the vascular wall, establishing a true secondary growth. In arterioles, the finding of tumor emboli breaking through the wall into the perivascular tissue was frequent. A striking example is offered by Figures 11 and 12, which represent serial sections of the same tumor embolus. Only a few tumor cells are visible in an extravascular position in Figure 11, while the lumen of the arteriole is plugged with tumor cells. Figure 12 shows their breaking through the wall into the surrounding tissue. It is reasonable to assume that the cells found in an extravascular position are derived from the obliterating embolus in the lumen of the vessel. Numerous other instances similar to this were found, and in all cases the rupture of the wall followed the complete obliteration of the lumen by tumor cells. Similar findings have been reported by Crabb [8] in a transplantable sarcoma of the hamster. In larger arteries, the cells would break the elastica interna, proliferate in the wall of the artery, and subsequently break through the elastica externa, invading the perivascular tissue. Mitotic activity was found in the walls of the arteries.

6. *Extravascular Growth of Viable Tumor Emboli.*—The emboli arrested in the capillaries grew beyond the endothelial wall, and in most instances the tumor cells invaded the alveolar lumen. In other instances the tumor cells grew along the perivascular tissue spaces of both veins and arteries, toward the center of the lobe. From arterioles the cells would grow along the perivascular tissue spaces, invading the alveolar structures only in late stages. The differentiation between secondary growths arising from the arterioles and those originating from capillaries presented some difficulty, especially the differentiation between a capillary growth growing backward in respect to the blood flow along perivascular tissue spaces [9] and an arterial growth extending downward along the perivascular tissue spaces toward the capillaries. The diagnosis was made on two features: (*a*) the finding on serial sections of a tumor embolus in the lumen of the arteriole and (*b*) the observation that growths arising from arterioles decreased in size as they extended downward along the tissue spaces, while, on the contrary, capillary growths were large at the periphery



Figs. 11 and 12.—Serial sections of a tumor cell embolus in a pulmonary arteriole. Note that there are only a few tumor cells in an extravascular position in Figure 11 (left); also note the breaking through the wall on Figure 12 (right). Hematoxylin and eosin; reduced ½ from mag. × 600.

¶ Baserga, A.: Unpublished data.

and decreased in size as they grew backward to the center of the lobe along the perivascular tissues. The final appearance of each of the two types of growth was characteristic. The capillary growths were solid, located as a rule at the periphery of the lobe, especially underneath or near the pleural surface, and large or medium-sized vessels were absent. Arteriolar growths were generally centered around an arteriole and the accompanying bronchus and were usually located in the center of the lobe, although they could also be found at the periphery, but not underneath the pleural surface.

The number of metastases arising from arterioles and from capillaries was counted; 40 mice, selected at random, with metastases were used for this count. The lungs of these 40 animals had a total of 684 metastatic growths (microscopic count), of which 456 (66.6%) originated from the capillaries, 35 (3.3%) from arteries, and 181 (28.2%) from arterioles. In 12 instances (1.9%) it was not possible to decide where the growth originated.

7. *Growth of Metastases.*—Once perivascular growth was established, the tumor cells, as we mentioned above, proliferated and extended, so that in late stages (usually in mice dying 35 or more days after the implant) the metastases could be as large as 5 mm. in diameter.

## COMMENT

C57 black mice, bearing a transplantable subcutaneous tumor, show a high incidence of blood-borne metastases in the lungs. If allowed to survive for two weeks or more after the implant, 100% of the animals have metastases in the lungs, the number of metastatic growths per mouse ranging from a few microscopic metastases to from 30 to 50 gross secondary growths. Metastases were not found in other organs in this series.

The finding of tumor emboli, lying free in the lumen of arterioles and capillaries of the lung and for the most part consisting of from a single to a maximum of seven cells, seems to indicate that the emboli spontaneously released by the primary growth consist of only a few cells. This indirectly supports the observation of Coman and associates [1] that single cells or small clumps are likelier to produce metastases than are large emboli. The possibility that postmortem manipulation may have dislodged tumor fragments and forced them for some distance along blood vessels [17] has been taken into consideration. However, our technique in removing, fixing, and sectioning the lungs was such as to avoid any manipulation or trauma of the organs; furthermore, in one instance a tumor embolus lying free in an artery was found in a lung without any gross or microscopic metastasis, though metastases were found in the contralateral lung. These observations are at variance with the results obtained by Watanabe [10] and by Weil [11] and with some autopsy reports.[17]

In our material, tumor emboli were often seen to lodge in the pulmonary arterioles by adhering to the endothelium, and not because of a disproportion between their size and the caliber of the blood vessels, whereas the capillaries were plugged at once by the emboli. The obliteration of the lumen of the arterioles was brought about by the subsequent formation of a thrombus surrounding the clump of tumor cells. Thereafter, the tumor cells proliferate into the lumen of the vessels and eventually invade and replace the thrombus. Thus, the blood clot itself does not seem to inhibit the growth of tumor cells.# In fact, it seems to offer a good medium for the growth of these cells and to be actually induced by the embolus. This thromboplastic property of tumor cells has been previously reported by Lawrence and co-workers.[23]

The endothelial barrier of the arterioles offers some resistance to the passage of tumor cells; there is perforation or infiltration of the wall only after the tumor cells have proliferated and almost filled the lumen of the blood vessel.

It is of interest that these findings on spontaneous blood-borne metastases have

# References 5, 13, and 22.

confirmed the results obtained by Coman and associates [1] and by Warren and Gates [9] with injection into the circulation of a suspension of tumor cells; i. e., that metastases arise more frequently from tumor emboli lodged in the capillaries than from those arrested in the arterioles. Histological studies on serial sections enabled us to determine that two-thirds of the metastatic growths in our material originated from capillaries.

Finally, it appears from our material that embolic tumor cells are capable of proliferating in the lumen of the blood vessels at the site of arrest, as shown by the frequency of mitotic figures, both in arterioles and in capillaries.

It seems that in order to establish a true metastatic growth, the cells must be able to proliferate in the lumen of the pulmonary vessels and to pass through the endothelial barrier. This had been already pointed out by Haaland,[24] and later by Takahashi.[8] The latter, using the method of injecting a tumor cell suspension into the tail vein of mice, tested various transplantable tumors and found that cells of tumors which never grew in the lung showed profound degenerative changes in the lumen of the pulmonary vessels, while cells of tumors which would eventually grow showed signs of proliferation. Takahashi [8] thought that the obstacle to the establishment of a true metastatic growth was presented by the endothelial barrier, and that once having passed such barrier, the tumor cells would grow undisturbed. In our experiment nonviable tumor emboli are emboli which fail to proliferate and grow at the site of arrest in the lumen of the blood vessels. This experimental evidence has been anticipated by Willis.*

> The critical period for embolic tumour cells is the period intervening between embolic lodgement and the attainment of an extravascular position by the proliferating cells. When the position is attained, the cells occupy once again their wanted relation to a connective tissue stroma. . . . The most precarious phase in the genesis of a metastatic growth is that of post-embolic intravascular sojourn of the tumour cells in the new environment.

* Willis,[17] p. 47.

## SUMMARY AND CONCLUSIONS

C57 black mice, bearing a subcutaneous transplant of an anaplastic carcinoma (T150), showed a high incidence of blood-borne metastases in the lungs. The histological findings are summarized and conclusions regarding the metastatic behavior of tumor T150 are drawn.

Tumor emboli spontaneously released by the primary growth are most likely to consist of single or a few tumor cells.

Such tumor emboli lodge in the pulmonary vessels either by adhering to the endothelium of arterioles or by "plugging" the lumen of capillaries. In arterioles, their arrest is not due to a simple "plugging" of the lumen.

In arterioles, the spread of tumor cells beyond the endothelial wall appears to be preceded by a phase of intraluminal growth. Signs of intraluminal growth were also detected in capillaries, though in this last case the possibility that occasional metastases might arise directly from extravasated tumor cells cannot be ruled out.

In a few instances regressing and degenerating tumor cell emboli were found in an intravascular position. No instance of regressing or degenerating extravascular growths was recorded.

Metastases originating from capillaries are more frequent than metastases originating from arterioles, the ratio being 2:1.

The arteriolar walls can be invaded from the inside, and in arteries the tumor cells are capable of proliferating in the wall between the two elastic membranes.

Mrs. Joline Ozanne prepared the histological material, and Mr. T. Scanlon, of the Chicago Medical School, prepared the photomicrographs.

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# BRONCHIOLAR (ALVEOLAR CELL) TUMORS

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THE PECULIAR pulmonary lesion which has been referred to as pulmonary adenomatosis, alveolar cell tumor, and alveolar cell carcinoma is still the subject of considerable controversy. Unanimity of opinion is lacking in regard to its being a true neoplasm and as to whether it originates from bronchi,[1] bronchioles,* or alveolar cells.† The lesion differs from other primary pulmonary neoplasms in that there is no demonstrable focus of origin from a bronchus and in that the distribution of mucin-forming cells is such that they form a lining for numerous alveoli.

## TERMINOLOGY

The large number of terms which have been used in referring to these tumors is ample proof of the existence of confusion as to their exact nature, their morphologic features, and their histogenesis. The following terms have been used in referring to these pulmonary neoplasms: alveolar cell tumor of lungs; primary multiple alveolar cell tumor; benign alveolar cell tumor of lungs; pulmonary alveolar adenomatosis; epithelization of alveolar walls; diffuse epithelial hyperplasia; pulmonary mucous epithelial hyperplasia; adenomatosis; adenoma-like tumor of lungs; multiple pulmonary adenomatosis; benign pulmonary adenomatosis; malignant pulmonary adenomatosis; alveolar cell cancer; alveolar cell carcinoma; carcinoma of alveolar epithelium; carcinomatoides alveogenica multicentrica; carcinosis; diffuse lung carcinoma; diffuse primary alveolar carcinoma; diffuse primary alveolar epithelial carcinoma; multicentric alveolar cell carcinoma; multiple nodular carcinoma; papillary gelatinous adenocarcinoma; mucocellular papillary adenocarcinoma; diffuse alveolar cell carcinoma; diffuse bronchiolar carcinoma; solitary bronchiolar carcinoma; multiple bronchiolar carcinoma; multiple circumscribed bronchiolar carcinoma; malignant adenomatosis, and cancerous pulmonary adenomatosis.

At the present time origin of these tumors from the epithelium of terminal bronchioles seems most probable. Consequently, the terms of choice are considered to be bronchiolar tumor, bronchiolar adenoma, and bronchiolar carcinoma.

## MORPHOLOGY

Benign (nonmetastasizing) and malignant (metastasizing) forms occur. The former are referred to as bronchiolar adenomas and the latter as bronchiolar carcinomas. Owing to the fact that most of the carcinomas, including many that have metastasized, are well differentiated (Figs. 11 and 20), it may be difficult to distinguish between benign and malignant forms in the absence of vascular invasion or metastasis. Nevertheless, the variations in clinical course, cellular pleomorphism (Figs. 9 and 12), and the occurrence of metastasis justify differentiation between two forms of the tumor.

In both the benign and the malignant tumors the condition is frequently not recognized until there is extensive involvement of both lungs. On the basis of the gross appearance of the lungs, diffuse [6] and multiple nodular [7] (Fig. 1) varieties are recognizable. In addition, combination forms (Fig. 3) are relatively frequent.

The diffuse form of bronchiolar adenoma frequently has a gross appearance like that of lobar pneumonia in the stage of gray hepatization. It has also been described as being

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* References 2 and 3.

† References 4 and 5.

similar to organizing and lipid pneumonia. Mucin may be present in sufficient amounts to be recognized as viscid, translucent material, and small cystic spaces are occasionally present. The high mucin content may lead to confusion of the gross appearance with that of pneumonia caused by Friedländer's bacillus, or Pneumococcus Type III. The striking microscopic features are numerous alveoli lined with epithelial cells and marked interstitial fibrosis and lymphocytic infiltration (Figs. 2 and 7). Frequently serial sections from different portions of affected pulmonary tissue demonstrate continuity between tumor cells lining alveoli and the epithelium of respiratory bronchioles or alveolar ducts (Figs. 18, 19, and 20). The epithelial tumor cells are uniform in size and staining reaction and may be ciliated (Figs. 5, 8, and 18) and form mucin. The ciliated epithelial cells are usually few in number and are identifiable only in well-preserved tissue by careful and prolonged examination. The mucin is both intracellular and intra-alveolar in location. It frequently completely fills the alveolar spaces (Figs. 2, 4, 7, and 18) and flattens the columnar tumor cells.

The nodular form of bronchiolar adenoma is characterized grossly by the presence of multiple gray or yellowish-gray nodules, 2 to 8 mm. in diameter (Fig. 1). No single tumor nodule is distinctive because of greater size, and no bronchi are primarily involved. Microscopically the cells are like those in the diffuse form and line alveoli in a similar manner. Serial sections usually reveal continuity between tumor cells lining alveoli and the epithelium of respiratory bronchioles or alveolar ducts in widely separated nodules.

Bronchiolar carcinoma also occurs in diffuse and nodular and in combination forms (Fig. 3). The gross appearance of the lung is similar to that in bronchiolar adenoma except that the tissue is usually less firm and gelatinous material is less conspicuous. The microscopic characteristics of the malignant tumor are similar to those of the benign tumor. Continuity between tumor cells lining alveoli and the epithelium of respiratory bronchioles or alveolar ducts (Figs. 18, 19, and 20) can be demonstrated at multiple sites. The tumors tend to be well differentiated, but poorly differentiated tumors occur. Examination of multiple sections will usually show, even in the well-differentiated tumors, considerable variation in cellular morphology. Thus, in some regions the cells are of regular mucin-forming types (Figs. 7, 11, and 16), like those of the bronchiolar adenoma, while in other locations the irregularity of tumor glands, abnormal nuclei, and mitotic figures are characteristic of malignant tumors (Figs. 4, 9, 10, and 12). Ciliated tumor cells can be demonstrated in many instances but are not constantly present and are fewer in number than in the benign tumors. The alveolar spaces more frequently contain projecting papillae of tumor (Fig. 14) and are less markedly distended with mucin than is true in benign tumors. In all probability there are tumors of this type without sufficient differentiation to be characterized by either cilia or mucin formation. Intrapulmonic lymphatic and blood vascular invasion is seen in the malignant tumors but is not common. Metastases, when present, are usually limited to the peribronchial and peritracheal lymph nodes but occasionally are distant, with involvement of brain, liver, and other organs.

The similarity between bronchiolar tumors and secondary carcinoma in the lungs [3] ordinarily requires the exclusion of other primary sites of carcinoma before a diagnosis of bronchiolar tumor is considered to be established.[9] It should be noted that in secondary carcinoma in the lung the tumor cells may line alveolar spaces, may be of goblet type, produce sufficient mucin to fill alveolar spaces, and show little cellular pleomorphism (Fig. 22). Occasionally in well-differentiated mucinous carcinoma secondary in the lung it may be possible to demonstrate continuity between the tumor cells lining alveoli and similar normal bronchiolar epithelium (Fig. 23).

Tumors originating from bronchial mucous glands [10] produce mucin but can be dis-

tinguished from those originating from bronchiolar epithelium by the absence of cilia.

The presence of ciliated tumor cells is the only anatomic feature of primary bronchiolar tumors that has not been noted in other primary or secondary tumors of the lung.

### RELATION TO ANIMAL TUMORS

The bronchiolar tumors of humans are also of interest because of morphologic similarities, particularly of the benign type, to pulmonary lesions of animals. This has led to speculation that the conditions are related and to unsuccessful attempts to transmit the tumors from humans to animals.‡ Pulmonary lesions, similar especially to the bronchiolar adenoma of man, have been described as developing spontaneously in sheep,[13] goats,[9] mice,[14] guinea pigs,[15] and horses.[16] In sheep the condition, which has been considered to be infectious, is referred to as *jagziekte*,[13] verminous pneumonia,§ epizootic adenomatosis,[19] and Montana progressive pneumonia.[21] Tumors of this type have also been produced in mice with carcinogenic agents.‖

The gross and microscopic features of the disease in animals are strikingly similar to those noted in human cases of bronchiolar adenomas. In sheep the lesions are usually extensive in both lungs; the normal pulmonary architecture is retained; the cells lining alveoli are of high columnar type, and proliferation and lymphocytic infiltration of interstitial tissue is marked (Fig. 25). The columnar epithelium is of mucin-forming type, but the alveoli are usually not distended with mucin. Continuity between cells lining alveoli and bronchiolar epithelium can be demonstrated.[13]

The exact cause of the spontaneous disease in animals is not known. It has been considered to be infectious and capable of epidemic spread. Many workers hold that it is of viral origin,[20] but this has not been established with certainty. Neither the possibility of its being a nonspecific response to irritants [15] nor the idea that it is a true tumor [24] can be excluded. The latter view is supported by one reported instance in which the pulmonary lesion of a sheep had metastasized to regional lymph nodes.[25]

### NATURE AND ORIGIN

At the present time most investigators are agreed that the pulmonary lesions in man are true neoplasms. As in other tumors, it is expected that there should exist an anatomically benign (nonmetastasizing) and malignant (metastasizing) form. A relation between the two forms was at first suggested in only a few reports ¶; now, however, they are logically considered as related tumors because of their morphologic similarities and because of the recorded cases of the benign form with focal malignant change.# The benign tumors are referred to as bronchiolar adenomas and the malignant ones as bronchiolar carcinomas. In the absence of metastasis or of pleural or vascular invasion, the differentiation is sometimes difficult.

Reasons for having considered the bronchiolar adenomas of humans as being inflammatory rather than neoplastic include the marked inflammatory reaction usually present (Figs. 2 and 7) and the morphologic similarities to the supposedly infectious disease (*jagziekte*) of animals (Fig. 25). In fact, sections of some bronchiolar adenomas, showing dilated alveoli lined with epithelium, marked interstitial fibrosis, and lymphocytic infiltration (Figs. 2 and 7), are suggestive of bronchiolectasis and chronic pneumonitis. An inflammatory basis, however, is not considered tenable because of the infrequency of bronchiectasis, the presence of cases with carcinomatous transformation (Figs. 4, 9, and 10), and the absence of inflammation in small tumors (Figs. 15, 16, 17, and 18).

The site of origin of these pulmonary tumors has been the subject of considerable dispute. The cells from which origin is possible are those lining alveoli, those of bron-

‡ References 11 and 12.

§ References 17 and 18.

‖ References 22 and 23.

¶ References 26 through 28.

# References 28 through 30.

chial glands, those lining bronchioles, and those lining bronchi.

The distribution of the tumor cells so that they line alveoli of course suggested origin from alveolar lining cells. This possibility calls to mind the unsettled question regarding the origin and nature of cells lining pulmonary alveoli. Many authors maintain that the alveoli are lined by a continuous epithelial layer *; others believe that the lining cells are epithelial but discontinuous †; some consider the cells to be mesenchymal and not epithelial,‡ and a few state that both epithelial and mesenchymal are present.§ Although the exact nature of normal cells lining alveoli has not been settled, there is no disagreement that in chronic pulmonary disease the interalveolar septa may have an investment of cuboidal or columnar cells (Fig. 24). Such cells, even if epithelial in type, differ from the tumor cell of bronchiolar tumors in that they are not ciliated and do not form mucin.

At present the cells of the bronchiolar tumors are considered to be epithelial, because of their cylindrical shapes, production of mucin, and ciliated free margins. The site from which these epithelial cells originate is not established with absolute certainty, but the accumulated evidence favors terminal bronchiolar cells. The following features of these tumors are considered to favor bronchiolar origin: topographic association of tumor nodules with bronchioles and not with bronchi; continuity between tumor cells lining alveoli and alveolar ducts or respiratory bronchioles; formation of mucin by tumor cells, and demonstration of ciliated tumor cells in many cases. The last finding is considered particularly significant, even though it is theoretically possible to explain the presence of ciliated tumor cells on the basis of metaplasia of mesodermal elements.[43]

Another controversial point about these tumors has been that of multicentric foci of development as opposed to origin from a single focus. The dissemination of tumor by lymphatics and/or air passage [44] is not considered adequate for the following: the involvement of all lobes of both lungs in nonmetastasizing forms of the tumor; the existence of multiple tumor nodules of similar size in one or all lobes of the lungs in both the benign and the malignant forms (i. e., the absence of any one nodule of significantly larger size than others), and the demonstrable continuity between bronchiolar or alveolar duct epithelium and tumor epithelium in many nodules. It is, therefore, considered most likely that these tumors ordinarily have multiple sites of origin in relatively widely separated respiratory bronchioles or alveolar ducts.

## CLINICAL FEATURES

The clinical signs and symptoms of these tumors are usually not diagnostic. The correct diagnosis prior to death or biopsy of the lung is suspected only after the exclusion of other, commoner cardiac and pulmonary diseases. The manifestations include cough, hemoptysis, cyanosis, dyspnea, pain in the chest, loss of weight, evidence of pleural effusion, clubbing of fingers, and signs of right ventricular enlargement. Careful evaluation of the history and laboratory examination may disclose the cough to be productive of sputum that is usually thick and viscid [44] because of its high content of mucin. It is also possible in the malignant form to demonstrate atypical (tumor) cells in sputum and bronchial washings.‖ The roentgenologic changes, which include diffuse mottling, due to small areas of increased density in all lobes of the lungs (Fig. 13), and cardiac enlargement (Fig. 6), are not diagnostic. The roentgenologic differential diagnosis includes cardiac failure (especially cor pulmonale) with passive hyperemia of lungs, atypical pneumonia, metastatic tumor, tuber-

* References 31 through 34.

† References 35 and 36. Palmer, D. M., in round-table conference, edited by Macklin.[35]

‡ References 37 through 41.

§ Sprunt, D. H., in round-table conference, edited by Macklin.[35] Ross.[42]

‖ References 45 through 47.

culosis, fungous infections, sarcoidosis, pulmonary fibrosis, and pneumoconiosis.

In reviewing the autopsied cases of carcinoma of the lung at Wesley Memorial Hospital between 1946 and 1954, the following eight bronchiolar tumors were encountered.

## REPORT OF CASES ¶

CASE 1.—A white man, 73 years old, had dyspnea for 11 months, accompanied by a 15 lb. (6.8 kg.) weight loss, but without cough, fever, or edema. Physical examination showed blood pressure 128/100. There were moist rales throughout both lungs and soft systolic murmurs at the cardiac apex and in the aortic area. There was cyanosis of the nail beds. Urinalysis and blood counts were normal, and the blood Kahn test was negative. Sputum examination revealed no acid-fast bacilli. X-rays showed diffuse, mottled increase in density throughout both lungs; the stomach and intestine were normal except for diverticula of the colon. The vital capacity was 48% of normal. The venous pressure was 22 cm. of water. Circulation times were, arm-to-lung, 14 seconds; arm-to-tongue, 30 seconds. A clinical diagnosis of cardiac failure due to pulmonary fibrosis was made. The patient was treated with low-salt diet, bed rest, digitoxin, oxygen, and aminophylline. The rales gradually increased, and in the fifth hospital week the patient suddenly became cyanotic and markedly dyspneic, had a convulsion, and died. The total duration of symptoms was one year. The clinical diagnosis was pulmonary fibrosis, generalized arteriosclerosis, and arteriosclerotic heart disease with fibrillation.

Autopsy (#2578, by R. J. Langenbach, M.D.) revealed diffuse bronchiolar adenoma involving both lungs, acute pneumonitis, moderate bronchiectasis, hypertrophy and dilatation of the heart, passive hyperemia of the liver and spleen, moderate arteriosclerosis of the coronary arteries and aorta, and arterial and arteriolar nephrosclerosis.

The right and left lungs each weighed 700 gm. The external surfaces were smooth except for a few adhesions near the apex of the left lung. All the lobes were firm and finely nodular (Fig. 1). Crepitation was markedly diminished. The cut surfaces showed fine nodules and exuded a moderate amount of gelatinous material. None of the nodules were connected with bronchi. Microscopic examination of all lobes of both lungs revealed a moderate increase in interstitial fibrous connective tissue which was infiltrated with lymphocytes and plasma cells (Fig. 2). The alveoli were lined by tall columnar epithelial cells, some of which were ciliated. Mucicarmine stains revealed mucin in the lumens of many of the alveoli and some of the lining cells. No invasion of vascular channels was noted, and no tumor was identified in regional lymph nodes or in other viscera. Extensive examination revealed a single area of continuity between the epithelium of a bronchiole and the tumor cells lining alveoli.

A complete autopsy was performed, with the exception of the cranium and its contents, and no other primary site of tumor was demonstrated.

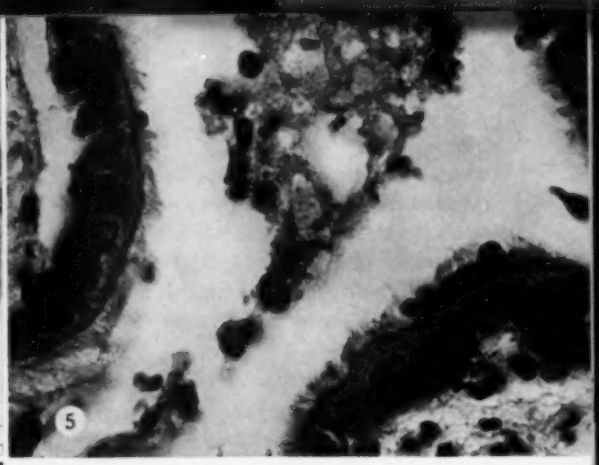
This case represents diffuse bronchiolar adenoma, cytologically benign.

CASE 2.—A white man, 69 years old, had malaise, weakness, and anorexia for seven months before death. Nonproductive cough appeared three months before death and was accompanied by dyspnea and occasional chest pain during the last month. He had lost 30 lb. (13.6 kg.) since the onset of his illness. On admission physical examination revealed blood pressure 107/70. The chest revealed coarse moist rales throughout. The laboratory data included leucocytosis, with a leucocyte count of 13,250, and a differential count showing 65 neutrophiles, 28 lymphocytes, and 7 eosinophiles. No acid-fast bacilli were found in the sputum on four occasions. First- and second-strength tuberculin (PPD, or purified protein derivative) tests were negative. Chest x-rays showed fine mottled densities in all lobes of both lungs. There was fluid at the left lung base. The mediastinum was moderately enlarged. Upper and lower gastrointestinal series showed no abnormality. The patient was treated with antibiotics and oxygen but failed to improve. On the 12th hospital day bronchoscopy showed no intrinsic bronchial lesions, and cytological examination of bronchial washings showed no identifiable tumor cells. The patient gradually became weaker and died on the 21st hospital day, eight months after onset of illness. The clinical diagnosis was metastatic carcinoma in lungs.

Autopsy (#3217, by T. K. Wong, M.D.) revealed diffuse and nodular bronchiolar carcinoma involving all lobes of both lungs, calcific aortic stenosis, chronic pulmonary emphysema, marked coronary arteriosclerosis, and adenocarcinoma of the prostate.

The right lung weighed 850 gm. and the left lung 750 gm. The pleural surfaces were smooth and shiny except for fibrous adhesions and a small amount of fibrinous exudate on the right. The crepitation of the lungs was increased, owing to the nodules. The nodules appeared on the pleural surfaces as pale gray and projected slightly. On section the nodules measured up to 1.5 cm. in diameter and were poorly demarcated from the surrounding lung (Fig. 3). No bronchial involvement by tumor was noted. Microscopically the tumor was composed of tall columnar epithelium, which lined alveoli and produced mucin. In many regions the tumor glands and cells were pleomorphic (Fig. 4); in others the tumor cells were regular and ciliated (Fig. 5). Similar tumor was

¶ Cases 3 and 5 have previously been reported.[28]



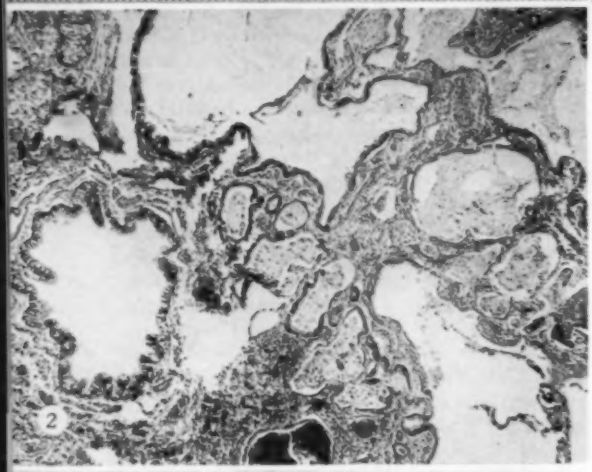


Fig. 1 (Case 1).—Bronchiolar adenoma. Multiple discrete tumor nodules beneath visceral pleura.

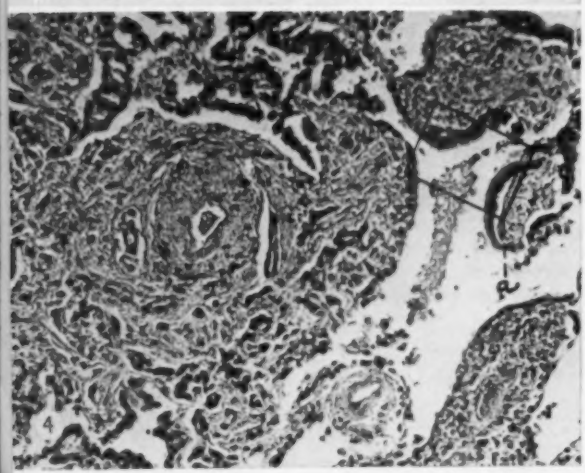
Fig. 2 (Case 1).—Bronchiolar adenoma. Alveoli containing mucin are lined with columnar tumor cells. Interalveolar septa are thickened. Note dilated epithelium-lined alveoli resembling bronchiectasis. Reduced 3/10 from mag. × 50.

Fig. 3 (Case 2).—Bronchiolar carcinoma originating in bronchiolar adenoma. Cut surface of lung showing diffuse and nodular involvement.

Fig. 4 (Case 2).—Bronchiolar carcinoma arising in bronchiolar adenoma. Pleomorphic glands and cells on left; regular columnar tumor cells on right; reduced 3/10 from mag. × 140. Location of Figure 5 is indicated at *a*.

Fig. 5 (Case 2).—Bronchiolar carcinoma arising in bronchiolar adenoma. Ciliated tumor cells lining alveoli in benign portion of tumor; reduced 3/10 from mag. × 860.

Fig. 6 (Case 3).—Bronchiolar adenoma with focal malignant change. Cardiac enlargement and changes in lungs are suggestive of cardiac failure rather than of primary tumor.



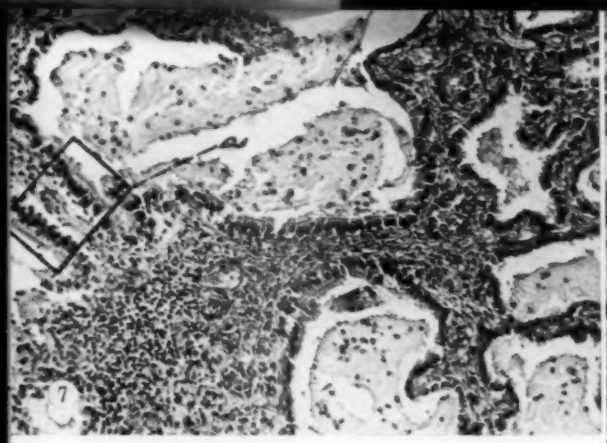


Fig. 7 (Case 3).—Bronchiolar adenoma with focal malignant change. Alveoli are distended with mucin and lined with columnar epithelium. Interstitial connective tissue is markedly increased in amount and infiltrated with lymphocytes. Reduced 3/10 from mag. × 500. Location of Figure 8 is indicated at *a*.

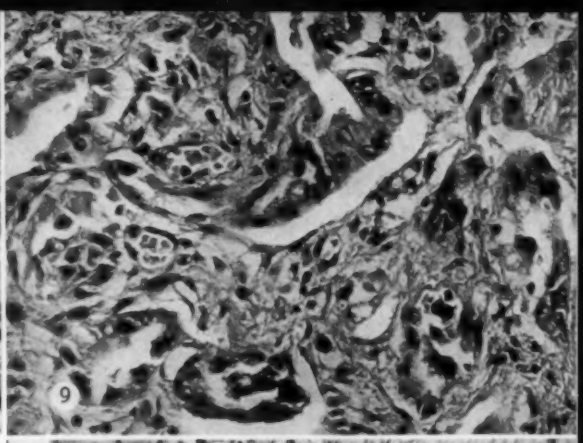
Fig. 8 (Case 3).—Bronchiolar adenoma with focal malignant change. Adjacent alveoli are lined with ciliated mucin-forming tumor cells. Reduced 3/10 from mag. × 1,200.

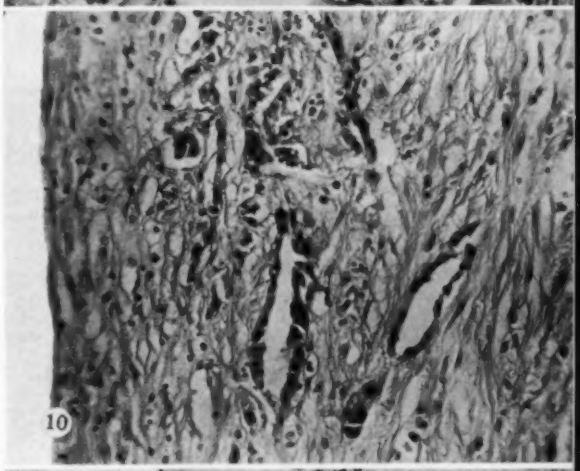
Fig. 9 (Case 3).—Bronchiolar adenoma with focal malignant change. Atypical tumor glands and cells in nodule from lower lobe of right lung; reduced 3/10 from mag. × 690.

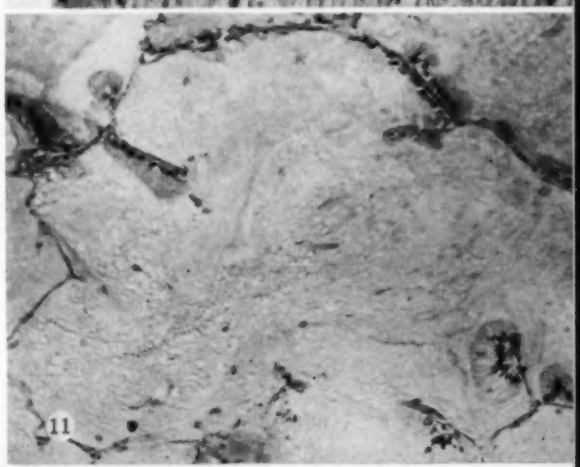
Fig. 10 (Case 3).—Bronchiolar adenoma with focal malignant change. Visceral pleura of lower lobe of right lung is invaded by atypical tumor glands. Reduced 3/10 from mag. × 500.

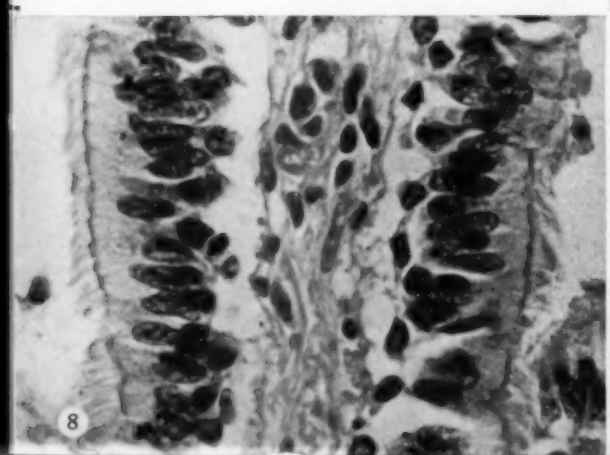
Fig. 11 (Case 4).—Bronchiolar carcinoma. Alveoli are distended with mucin and lined by tumor cells of goblet type. Reduced 3/10 from mag. × 137.

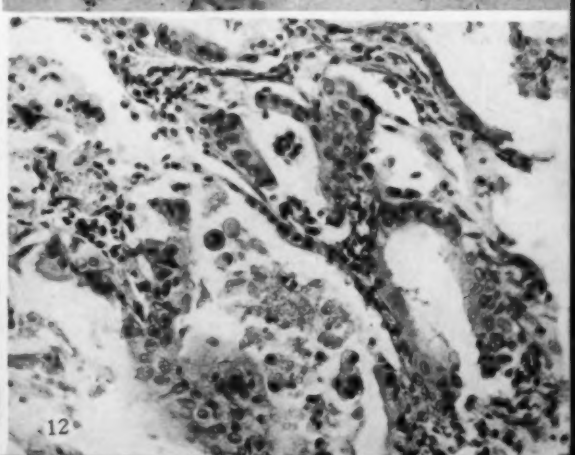
Fig. 12 (Case 4).—Bronchiolar carcinoma. Tumor cells lining alveoli show moderate pleomorphism. Reduced 3/10 from mag. × 270.











present in several bronchopulmonary lymph nodes. Serial sections of the nodules showed continuity between bronchiolar epithelium and tumor lining alveoli.

The prostate was nodular and increased in size. Microscopic sections showed, in one region, a tiny adenocarcinoma which invaded perineural sheaths. However, this tumor did not produce mucin and was not considered to be a possible primary site of the tumor in the lungs. A complete autopsy was performed, and no other primary site of tumor was identified.

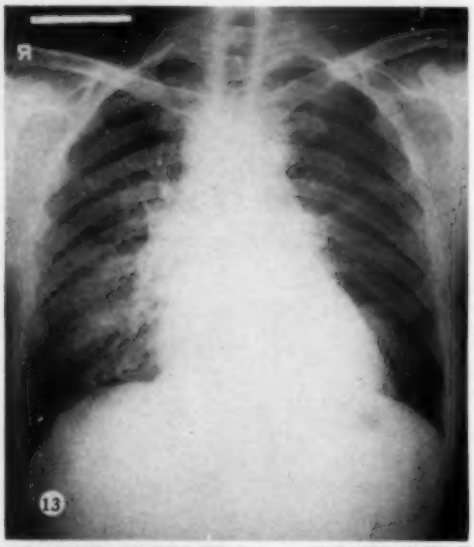



Fig. 13 (Case 5).—Bronchiolar carcinoma. Diffuse pulmonary mottling due to increased density of tumor nodules.

Fig. 14 (Case 5).—Bronchiolar carcinoma. Tumor cells line alveoli and form papillae projecting into alveolar spaces. Reduced 3/10 from mag. × 60.

Fig. 15 (Case 6).—Bronchiolar adenoma. Subpleural tumor, 3 mm. in maximum measurement, was an incidental autopsy finding. Reduced 3/10 from mag. × 25. Location of Figures 16 and 18 are indicated at *a* and *b*.

Fig. 16 (Case 6).—Bronchiolar adenoma. Mucin-forming epithelium lines alveoli. Fibrosis and inflammatory reaction are not present. Reduced 3/10 from mag. × 370. Location of Figure 17 is indicated at *a*.

Fig. 17 (Case 6).—Bronchiolar adenoma. Ciliated tumors cells lining alveolus; reduced 3/10 from mag. × 1,300.

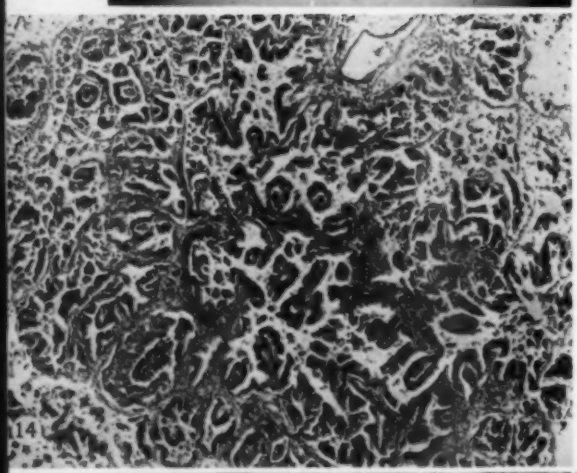
Fig. 18 (Case 6).—Bronchiolar adenoma. Normal epithelium of respiratory bronchiole (*R. B.*) is continuous with ciliated tumor cells lining alveolar ducts (*A. D.*) and alveoli (*A.*). Reduced 3/10 from mag. × 380.

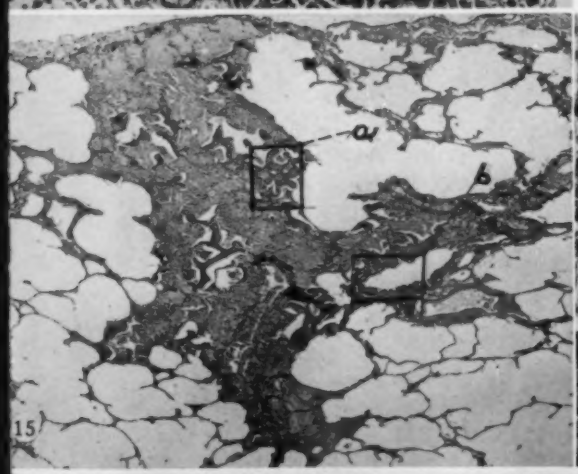
Fig. 19 (Case 7).—Bronchiolar carcinoma. Epithelium of respiratory bronchiole (*R. B.*) is continuous with tumor cells which line alveolar duct (*A. D.*) and alveoli. Reduced 3/10 from mag. × 50. Location of Figure 20 is indicated by the parallelogram.

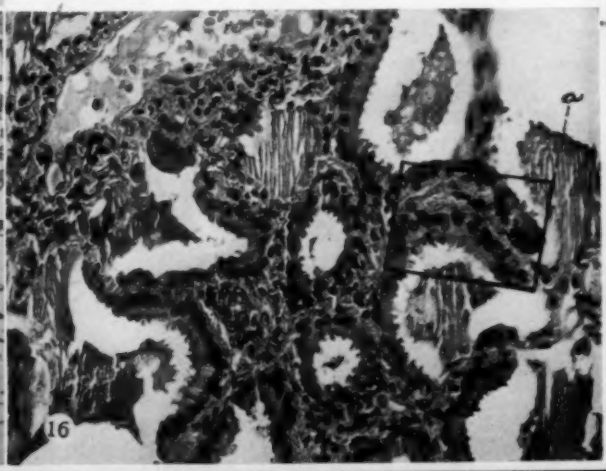
Fig. 20 (Case 7).—Bronchiolar carcinoma. Tumor cells lining alveolar duct and alveoli show little pleomorphism. Reduced 3/10 from mag. × 120.

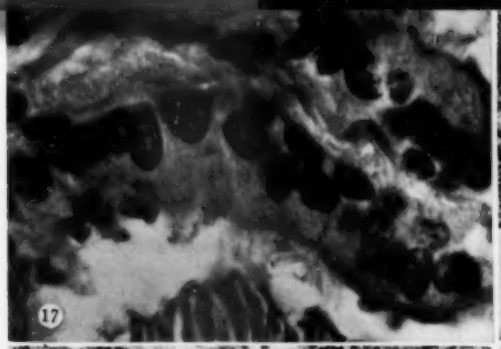
Fig. 21 (Case 8).—Bronchiolar adenoma. Alveoli are lined by tumor cells. Neutrophiles within alveoli are due to terminal bronchopneumonia. Reduced 3/10 from mag. × 80.

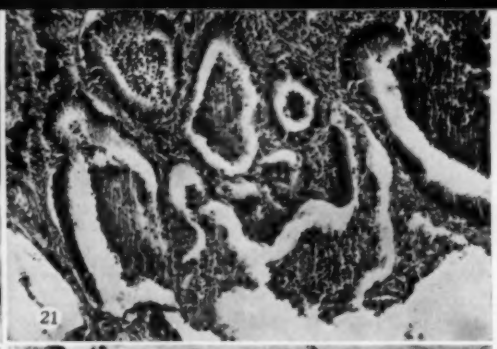
Fig. 22 (Case of primary carcinoma of pancreas with metastasis to lungs).—Nonciliated tumor cells of goblet type line alveoli. Mucin fills alveolar spaces. Reduced 3/10 from mag. × 245.

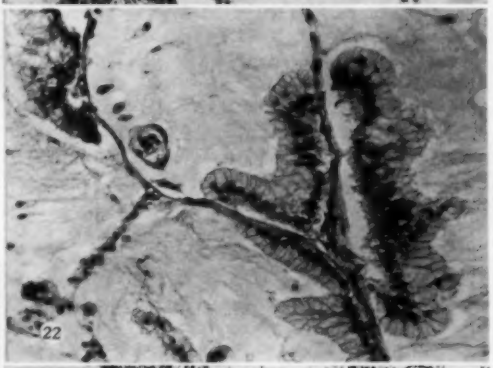


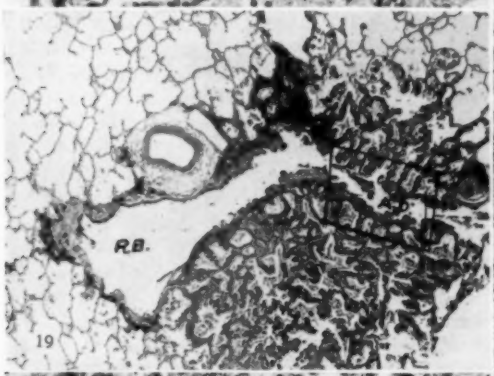



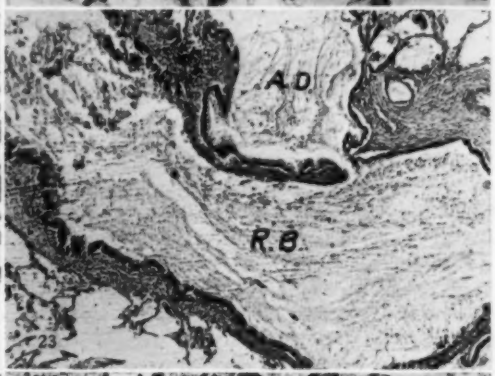



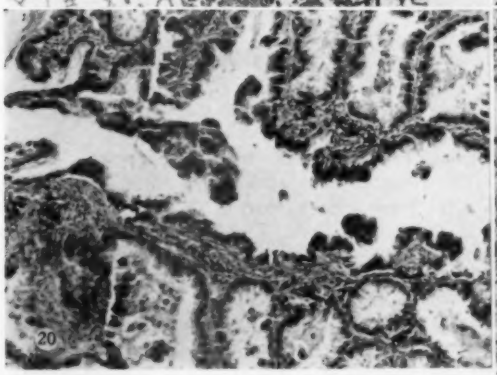


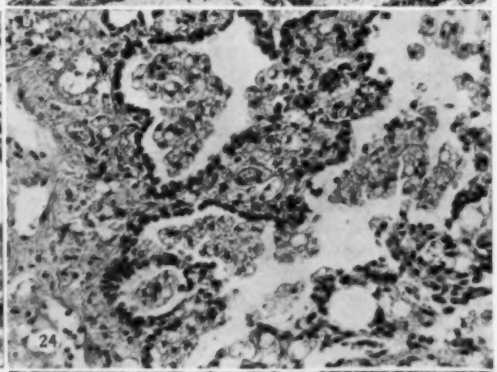








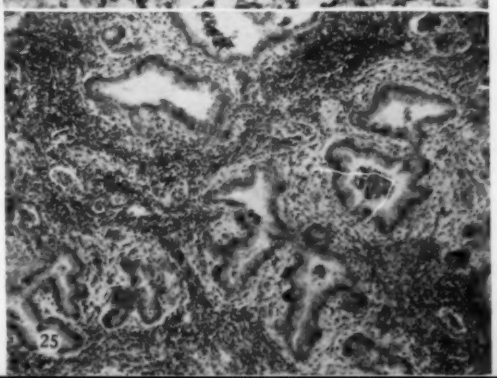


Fig. 23 (Case of primary carcinoma of pancreas with metastases to lungs).—Normal epithelium of respiratory bronchiole (*R. B.*) appears to be continuous with tumor cell portion of it and alveolar duct (*A. D.*). Reduced 3/10 from mag. × 65.

Fig. 24 (Case of lipid pneumonia).—Columnar metaplastic cells lining alveoli are epithelial-like but do not have cilia and do not form mucin. Reduced 3/10 from mag. × 245.

Fig. 25 (Case of *jagziekte* in sheep).—Lung shows marked fibrosis, lymphocytic infiltration, and epithelial-lined alveoli. Reduced 3/10 from mag. × 122.

This case is considered to be bronchiolar carcinoma originating in a bronchiolar adenoma.

CASE 3.—A white woman, 51 years old, had noted gradually increasing exertional dyspnea for one year, with development of nonproductive cough five months before her first admission to Wesley Memorial Hospital. Physical examination showed blood pressure 120/80. There was cyanosis, cardiac enlargement to right and left, signs of pulmonary congestion, pleural fluid, ascites, and dependent edema. The laboratory data included albuminuria. An electrocardiogram revealed marked right heart strain, and a chest x-ray showed cardiac enlargement and pulmonary congestion (Fig. 6). After treatment with oxygen, digitoxin, and diuretics the patient's symptoms became less marked, but cyanosis was still present on discharge, on the 43rd hospital day. A month later readmission occurred because of cough, dyspnea, and edema. Physical examination showed progressive evidence of heart failure, with marked edema. In spite of treatment the patient died on the third hospital day, 14 months after onset of illness. The clinical diagnosis was Ayerza's disease.

Autopsy (#2099, by C. I. Fisher, M.D.) revealed hypertrophy and dilatation of the right ventricle and right atrium, marked sclerosis of pulmonary arteries and arterioles, bilateral acute fibrinous pleuritis and acute bronchopneumonia, diffuse bronchiolar adenoma involving both lungs and invading the interstitial tissue and visceral pleura of the lower lobe of the right lung, herpes zoster of the skin on the left side of the thorax, moderate arterial and arteriolar nephrosclerosis, ascites (1,400 cc.), and moderate edema of the lower extremities.

The heart weighed 305 gm. The hypertrophy was limited to the right atrium and right ventricle. The wall of the right ventricle, in spite of marked dilatation, measured 8 mm. in thickness.

The right and left lungs weighed, respectively, 800 and 600 gm. All lobes of both lungs were increased in size and consistency, with reduced crepitation. In the posterior portion of the lower lobe of the right lung there were two poorly circumscribed, firm, spherical, pale yellowish-gray nodules, with maximum measurements of 0.9 and 2.0 cm. These nodules showed no connection with bronchi. Microscopic sections of all lobes of both lungs showed an impressive increase in the amount of interstitial fibrous connective tissue, in which there were many lymphocytes and plasma cells (Fig. 7). The alveoli were lined by tall columnar epithelial cells, many of which were ciliated (Fig. 8). Some of these cells, as well as the alveoli, contained material which stained positively for mucin by the mucicarmine method. The two nodules in the lower lobe of the right lung were made up of richly cellular, invasive neoplasm which formed glands and mucin (Figs. 9 and 10). Throughout the lungs, the microscopic features were those of bronchiolar adenoma, while in the nodules in the lower lobe of the right lung, the changes were those of adenocarcinoma. With serial sections of several lesions near bronchioles, continuity between tumor epithelium and normal bronchiolar epithelium was noted.

A complete autopsy was performed, and no other primary site of tumor was demonstrated.

This case is reported as diffuse bronchiolar adenoma, with two foci of malignant change.

CASE 4.#—A white diabetic woman, 59 years old, with hypertensive cardiovascular disease for seven years, was admitted to Cook County Hospital with chest pain, dyspnea, and cough of four days' duration. Physical examination revealed temperature 101.2 F., pulse 124, respirations 32, and blood pressure 146/88. There were moist rales over both lung bases. The heart was enlarged, and there was a gallop rhythm. The laboratory data included those of diabetes, with slight acidosis. The white blood cell count was 15,100. The chest x-ray showed no significant abnormality. The patient responded to the usual treatment for diabetes but on the 16th hospital day had a severe insulin reaction and died the next day. The clinical diagnosis was diabetes mellitus and myocardial infarct.

Autopsy (#1406, by Geoffrey Kent, M.D.) revealed marked coronary arteriosclerosis, with a recent thrombus of the right coronary artery, an old thrombus of the circumflex branch of the left coronary artery, and recent myocardial infarct of the interventricular septum and posterior wall of the left ventricle; hypertrophy and dilatation of heart, and bronchiolar carcinoma, lower lobe of right lung.

The total weight of the lungs was 1,250 gm. The pleural surfaces were smooth and shiny. There were several 0.4 to 0.6 cm. calcified subpleural nodules, some of which had caseous centers. Similar nodules were noted in the bronchopulmonary lymph nodes. The lower lobes of both lungs were dark red and increased in consistency. In the lower lobe of the right lung a roughly spherical cavity, 2.5 cm. in diameter, was filled with gelatinous gray material. The surrounding lung was firm and pale gray. Microscopic sections of this nodule showed that it was composed of richly cellular epithelial tumor lining alveolar spaces (Figs. 11 and 12). Some of the glands and tumor cells contained mucin. A few of the epithelial cells were ciliated. Some of the cells were slightly larger than others and showed nuclear pleomorphism with occasional bizarre nuclear forms (Fig. 12). A complete autopsy was performed, with the exception of the cranium

# This case is published by the courtesy of Hans Popper, M.D., Cook County Hospital, Chicago.

and its contents, and no other primary site of tumor was identified. No communication with bronchi or bronchioles was noted.

This case is reported as bronchiolar carcinoma.

CASE 5.—A white man, 45 years old, was admitted because of pain in the chest on coughing, of nine months' duration. During the month before admission there had been dyspnea, hemoptysis, and anorexia. There had been a 25 lb. (11.3 kg.) weight loss. Physical examination showed temperature of 101 F. and blood pressure 120/80. Several 2 cm. nontender supraclavicular lymph nodes were noted. There was dullness at both lung bases, with bronchial breathing and a friction rub at the left base. The heart was not enlarged, and there were no murmurs. Urinalysis and blood counts were normal except for leucocytosis, with a leucocyte count of 30,500 and a differential count of 92 neutrophilic leucocytes and 8 lymphocytes. Seven sputum examinations and one guinea pig inoculation were negative for tuberculosis. A chest x-ray (Fig. 13) showed a fine mottled increase in density throughout both lungs, more in the central area. The superior mediastinum was large, and the heart shadow was 30% enlarged. Tuberculin, coccidioidin, and histoplasmin skin tests were negative. The patient was treated with penicillin and streptomycin and improved for two weeks. On the 14th hospital day he noted severe pain in the left side of the chest, with marked dyspnea, cyanosis, and tachycardia. These symptoms became severer, and he died two days later, 9½ months after onset of symptoms. The clinical diagnoses considered were sarcoidosis and miliary tuberculosis.

Autopsy (#2275, by F. A. Svec, M.D.) revealed bronchiolar carcinoma of the lung with invasion of pleura and intrapulmonary lymphatics and metastases in mediastinal and cervical lymph nodes and left adrenal gland; recent organizing thrombosis of peripheral branches of the pulmonary artery to the upper and lower lobes of the left lung; organizing infarcts in the corresponding lobes; hydropericardium (500 cc.); left hydrothorax (1,500 cc.); hypertrophy and dilatation of the heart, particularly of the right atrium and right ventricle (400 gm.); marked passive hyperemia of viscera, and slight arterial and arteriolar nephrosclerosis.

The right and left lungs weighed 1,150 and 900 gm., respectively. All lobes of both lungs contained many firm, pale yellow nodules, sharply circumscribed from surrounding pulmonary tissue and measuring from 0.1 to 1.5 cm. in maximum dimension. None of the tumor nodules was outstanding in size, and bronchial involvement could not be demonstrated. Microscopically the nodules were similar to each other. They were composed of richly cellular papillary epithelial tumor (Fig. 14), which in many places lined alveoli. Sections stained by the mucicarmine method revealed mucin within tumor cells and within alveoli lined by tumor cells. No cilia were identified. Serial sections of representative nodules failed to reveal continuity between tumor and normal bronchiolar epithelium. A complete autopsy was done, and no other primary site of tumor was identified.

This case is reported as multiple nodular type of bronchiolar carcinoma.

CASE 6.*—A white woman, 66 years old, was admitted with abdominal pain, fever, and vomiting of three days' duration. She had also noted a lump in the scar of a uterine suspension done seven years before. Physical examination revealed temperature of 102.4 F., pulse 116, blood pressure 160/60. The patient was dehydrated and appeared seriously ill. There was an irreducible mass at the site of the old abdominal incision. Laboratory data included mild acidosis and leucocytosis, with a leucocyte count of 15,000. An operation was performed on the day of admission, and a strangulated loop of colon was noted in the hernia sac. This loop had perforated, and there was an abdominal wall abscess. The abscess was drained and the loop of colon exteriorized as a colostomy. Postoperatively the patient gradually developed partial small intestinal obstruction and a fistulous tract between the incision and the small intestine. The blood urea nitrogen rose to 182 mg. per 100 ml. On the 79th hospital day another surgical procedure was performed, with lysis of multiple peritoneal adhesions and closure of the fistula. The patient had shock and anuria after the operation and died three days later.

Autopsy (#4108, by George F. Stevenson, M.D.) revealed multiple fibrous peritoneal adhesions with an old abscess of the anterior abdominal wall, nonbacterial thrombotic endocarditis, chronic pulmonary emphysema, acute tracheobronchitis, marked arterial and arteriolar nephrosclerosis, and bronchiolar adenoma.

The right and left lungs weighed, respectively, 270 and 230 gm. They showed no gross abnormality except for emphysema and bronchitis. In one of the lung sections a subpleural nodule less than 0.3 cm. in diameter was noted (Fig. 15). This nodule was unencapsulated and composed of tall columnar epithelial cells lining alveolar spaces (Fig. 16). These cells were uniform in size and shape and had medium-sized nuclei which were not pleomorphic. Some of the cells and some of the alveolar spaces contained material which stained positively with the mucicarmine stain. Some of the tumor cells were ciliated (Fig 17). There was continuity between the small bronchiole at the edge of the nodule and the tumor epithelium (Fig. 18). A complete autopsy

* This case is published by the courtesy of George F. Stevenson, M.D., St. Joseph Hospital, Chicago.

was performed, with the exception of the neck organs and cranial contents, and no other tumor was found.

This incidental finding is considered to be the smallest bronchiolar adenoma yet described.

CASE 7.—A white man, 63 years old, had dyspnea for 2½ years before death, accompanied by weight loss of 25 lb. (11.3 kg.). For 18 months he had noted chronic cough with production of large amounts of mucus. Seventeen months before death an exploratory thoracotomy was performed, and a large hard tumor had been palpated in the left lung, with numerous nodules scattered over the pleura. No biopsy specimen was taken. During the next month x-ray therapy was given, but the patient gradually became worse and was admitted to the hospital for terminal care. Physical examination at that time showed blood pressure 130/82. There were signs of fluid in the left chest. X-rays showed a large mass at the left hilus, with many mottled areas of increased density throughout the left lung. Laboratory examinations included a normal urinalysis, hemoglobin 14 gm. per 100 ml., red blood cell count 4,980,000, and white blood cell count 36,000, per cubic millimeter. The patient was treated symptomatically and died on the eighth hospital day, 2½ years after onset of symptoms. The clinical diagnosis was carcinoma of lung with metastases, and recent arterial embolus, right forearm.

Autopsy (#2981, by J. P. Pfaff, M.D.) revealed bronchiolar carcinoma involving both lungs, and liver, splenic capsule, omentum, and aortic and mediastinal lymph nodes.

The right lung weighed 750 gm. Palpation disclosed numerous firm nodules throughout all the lobes and a decreased amount of crepitation. The cut surfaces showed multiple gray nodules, measuring up to 0.5 cm. in diameter, scattered irregularly throughout the entire lung. All of the left lung that was found was a firm portion measuring 6.5 cm. in greatest diameter and 1 cm. in thickness. The cut surfaces were firm and gray, mottled with black. A primary tuberculous complex was found in the right middle lobe and peribronchial lymph node. The remaining mediastinal lymph nodes were moderately enlarged and firm and sectioned with moderate resistance, to reveal gray and black cut surfaces. A firm gray nodule was found near the surface of the right lobe of the liver. No bronchial involvement by tumor was noted. Microscopic examination of the right lung revealed alveoli lined by columnar epithelium (Figs. 19 and 20). These cells exhibited little pleomorphism and only a few mitoses. Mucicarmine stains revealed mucin in the alveolar lumens and in many of the lining cells. None of the cells were ciliated. The left lung showed numerous dilated bronchi, large amounts of fibrous connective tissue, and small numbers of alveoli lined by cells similar to those seen in the right lung. In many of the nodules in both lungs continuity between normal bronchiolar lining cells and tumor cells was noted (Figs. 19 and 20). Sections of the liver, splenic capsule, omentum, aortic and mediastinal lymph nodes, and the skin and subcutaneous tissue of the left thorax revealed tumor similar to that in the lung, except that more pleomorphism was present. A complete autopsy was performed, and no other primary tumor was found.

This case represents an example of the multiple nodular variety of bronchiolar carcinoma.

CASE 8.—A white woman, 83 years old, had nonproductive cough for 18 months, accompanied by weight loss of 15 lb. (6.8 kg.). She had noted weakness and fatigue for two weeks. Physical examination showed blood pressure 144/84. There were fine dry crackling rales throughout both lungs. The heart was not enlarged, and there were no murmurs. A urinalysis was normal. The hemoglobin was 11 gm. per 100 ml.; the red cell count was 3,800,000, and the white blood cell count 12,200, per cubic millimeter. The differential count of 100 cells showed 60 neutrophiles, 34 lymphocytes, 2 monocytes, and 4 eosinophiles. The blood Kahn test was negative. Sputum cultures were negative for tuberculosis and other pathogenic bacteria; one culture showed a light growth of Candida albicans. X-rays showed diffuse mottled increase in density throughout both lungs. There was deviation of the trachea to the right and elongation of the aorta. The patient improved with digitoxin, penicillin,

TABLE 1.—*Anatomic Changes in Bronchiolar Tumors*

| Case No. | Sex | Age | Character | Cilia | Mucin | Bronchiolar Continuity |
|---|---|---|---|---|---|---|
| 1 | M | 73 | Benign | + | + | + |
| 2 | M | 69 | Malignant transformation of benign | + | + | + |
| 3 | F | 51 | Focally malignant | + | + | + |
| 4 | F | 59 | Malignant | + | + | — |
| 5 | M | 45 | Malignant | — | + | — |
| 6 | F | 66 | Benign | + | + | + |
| 7 | M | 63 | Malignant | — | + | + |
| 8 | F | 83 | Benign | + | + | + |

TABLE 2.—*Clinical Data on Bronchiolar Tumors*

| Case No. | Sex | Age | Fever | Weight Loss, Lb. | Cough | Dyspnea | Edema | Sputum | Hemoptysis | Pain | Heart Size | X-Ray | Clinical Diagnosis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 73 | 0 | 15 | 0 | 11 mo. | 0 | 0 | 0 | 0 | ++ | Diffuse mottling | Heart failure, pulmonary fibrosis |
| 2 | M | 69 | 0 | 30 | 3 wk. | 1 mo. | 8 mo. | 0 | 0 | 0 | Normal | Diffuse mottling | Carcinomatosis |
| 3 | F | 51 | 0 | 15 | 5 mo. | 14 mo. | 4 wk | 0 | 0 | 0 | + | Passive hyperemia | Ayerza's disease |
| 4 | F | 59 | ..... | .. | ..... | ..... | Incidental autopsy findings | | | ..... | ..... | ..... | ..... |
| 5 | M | 45 | 9 mo. | 25 | 1 mo. | 1 mo. | 0 | "A little" | 1 mo. | 9 mo. | Normal | Diffuse mottling | Sarcoidosis or miliary tuberculosis |
| 6 | F | 66 | ..... | .. | ..... | ..... | Incidental autopsy findings | | | ..... | ..... | ..... | ..... |
| 7 | M | 63 | 1 wk. | 22 | 18 mo. | 2 yr. | 0 | "Much mucus" | 0 | 18 mo. | Normal | Mass, left hilus | Carcinoma of lung |
| 8 | F | 83 | 0 | 15 | 18 mo. | 3 wk. | 3 wk. | 0 | 0 | 0 | Normal | Diffuse mottling | Carcinoma of lung |

oxytetracycline (Terramycin), and dihydrostreptomycin treatment and was discharged on the 36th hospital day. She was readmitted two weeks later with increasing cough, now productive of small amounts of nonpurulent sputum. Physical and laboratory examinations were essentially unchanged. An electrocardiogram showed right bundle branch block and R-T changes consistent with digitalis effect. Treatment with bed rest, digifolin, meralluride U. S. P. (Mercuhydrin), and aminophylline had little effect, and the patient died on the 19th hospital day, 14 months after onset of symptoms. The clinical diagnosis was carcinoma of lung.

Autopsy (#2951, by W. E. Cape, M.D.) revealed diffuse bronchiolar adenoma involving both lungs, chronic interstitial pneumonitis with focal fibrosis, bronchopneumonia and bronchiectasis of both lungs, passive hyperemia of the liver and edema of the lower extremities.

The right and left lungs weighed 600 and 400 gm., respectively. All lobes of both lungs had a uniform firmness. In some areas a slight nodularity was present. The lungs sectioned with increased resistance to reveal firm, diffusely consolidated gray cut surfaces with a few nodules in some areas. Dilated bronchi were also seen on the cut surfaces. No bronchial involvement by tumor was noted. Microscopic examination revealed extensive fibrosis between alveoli. In these areas numerous lymphocytes and moderate numbers of neutrophiles were present. The alveoli were lined almost entirely by columnar epithelium (Fig. 21), which was ciliated in numerous areas. Some cells had desquamated into the lumen. Some of the alveoli contained numerous neutrophiles. Mucicarmine stains revealed mucin in the lumens of the alveoli and in most of the columnar cells lining alveoli. No invasion of lymphatics or blood vessels were seen. One area of continuity between the lining cells of the alveoli and a bronchiole was noted. Careful examination revealed no metastatic tumor in regional lymph nodes or in other viscera. A complete autopsy was performed, and no other site of primary tumor was found.

This case represents a bronchiolar adenoma, cytologically benign.

## COMMENT

*Clinical Features.*—In this series of cases the clinical findings were those of progressive cardiac failure or diffuse pulmonary disease (Tables 2 and 3). Most of the patients had cough and dyspnea. All of the patients had weight loss, varying from 15 to 30 lb., which is uncommon in patients with uncomplicated cardiac failure. Peripheral edema, present in three patients, was associated with cardiac failure.

The sex distribution of the group of patients was equal, four men and four women. The youngest patient was 45 and the oldest 83. Of the others, two patients were in the sixth, three in the seventh, and one in the eighth decade. Of the six patients for whom a history was recorded, the only one who smoked at all smoked only six cigarettes daily (Case 1).

The production of sputum was noted in only two patients; one of these expectorated only "a little" sputum, while the other expectorated "frothy mucus" in large amounts. The relative sparsity of sputum is in marked contrast to that usually described in bronchiolar tumors, in which the patient is said to expectorate large amounts of slimy, frothy mucus. Thus, the diagnosis of bronchiolar tumor should not be excluded simply because expectoration is scant. Nevertheless, it is considered likely that careful questioning will elicit a history of expectoration of large amounts of sticky sputum in patients with bronchiolar tumors.

TABLE 3.—*Summary of Clinical Data on Six Cases of Bronchiolar Tumors*

| | |
|---|---|
| Dyspnea | 6 |
| Weight loss | 6 |
| Cough | 5 |
| Edema | 3 |
| Sputum | 2 |
| Pain | 2 |
| Fever | 2 |
| Hemoptysis | 1 |

Cardiac enlargement was present in two of the six cases and was principally of the right ventricle, probably related to increased pulmonary resistance. The x-rays in general showed diffuse mottling throughout all lobes of both lungs, like that seen in miliary tuberculosis, sarcoidosis, beryllosis, hemosiderosis, metastatic tumor, or pulmonary fibrosis. In one case a mass at the left hilus with multiple nodules in both lungs was noted.

The correct clinical diagnosis was not made in any of these cases. Metastatic carcinoma was considered in three cases, while pulmonary fibrosis, Ayerza's disease, sarcoidosis, or miliary tuberculosis were thought possible in the others.

One may conclude that the clinical diagnosis of bronchiolar tumors is difficult. The condition should be considered in a patient who has progressive cough, dyspnea, weight loss, and roentgen evidence of diffuse infiltration in the lungs. Production of large amounts of mucinous sputum is probably common but not essential for the diagnosis. The finding of tumor cells in the sputum should be helpful. In our experience this has not been common, perhaps because the tumor cells so closely resemble normal bronchial epithelial cells.

*Morphology.*—These eight cases fulfill the criteria for the diagnosis of bronchiolar tumor, each being characterized by the following: the presence of tall columnar epithelium lining alveoli; the presence of mucin in tumor cells and alveolar spaces, and the absence of primary tumor in bronchi or elsewhere in the body.

In addition, these cases show anatomic features (Table 1) which have not been emphasized in the extensive literature on the subject. The presence of ciliated tumor cells has previously been described in a few bronchiolar tumors,† but little significance has been attached to it. In the present series, six of the eight cases showed definite parallel rows of cilia on some or many of the tumor cells. Cilia were readily identified in the benign bronchiolar adenomas and in the well-differentiated bronchiolar carcinomas, but, as might be expected, cilia were uncommonly seen in the less well-differentiated tumors. The presence of cilia in these tumors is considered to be strong evidence that they originate from the ciliated epithelium of terminal, or respiratory, bronchioles.

The production of mucin has been generally accepted as one of the characteristics of bronchiolar tumors. In all of the present group of cases, abundant mucin was present in alveoli and in tumor cells. The production of mucin is considered to be additional evidence of origin of these tumors from mucin-producing epithelium, such as that which lines bronchioles.

† References 8, 9, and 28.

Continuity between normal bronchiolar epithelium and tumor was observed in six of our eight cases. Involvement of bronchiolar epithelium has been previously described‡ and would suggest origin from the involved bronchiole. However, since secondary tumors may produce similar bronchiolar continuity, this is considered only as consistent with bronchiolar origin.

The morphologic features of the tumors in the present group of cases, namely, cilia, mucin, bronchiolar continuity, and absence of primary tumor elsewhere in the body, support origin of these tumors from bronchiolar epithelium rather than from alveolar lining cells. Ciliated tumor cells are considered to be the most distinctive anatomic feature of this group of tumors.

## SUMMARY

Eight cases of bronchiolar (alveolar cell) tumors are reported. On the basis of morphologic criteria, three are classified as benign (bronchiolar adenoma), three as malignant (bronchiolar carcinoma), and two as benign with malignant changes.

The correct clinical diagnosis was not made in any of the cases. The commonest clinical signs and symptoms were dyspnea, weight loss, cough, and peripheral edema. None of the patients was noted to have coughed up gelatinous sputum. Nevertheless, because of the large amount of mucin within bronchioles and alveoli at the time of autopsy, it seems probable that careful clinical evaluation of patients with such tumors should disclose the production of large amounts of sticky, tenacious sputum.

The morphologic features in all eight cases include alveoli lined by columnar cells, presence of mucin within cells and alveoli, and absence of primary tumor elsewhere in the body. The apparent lining of alveoli by tumor cells and the continuity of tumor with bronchiolar epithelium were striking, but since these features may also be noted in secondary carcinoma in the lung, they are not considered diagnostic of bronchiolar tumors.

‡ References 2, 3, and 8.

Ciliated tumor cells were demonstrated in six of the eight cases and are considered as the most characteristic single anatomic feature of these tumors. In order to establish the diagnosis of bronchiolar tumor, particularly in the absence of ciliated tumor cells, it is considered essential to exclude the existence of an extrapulmonary primary tumor by complete autopsy.

Photomicrograph prepared from tissue furnished by Hadleigh Marsh, D.V.M., Bozeman, Mont.

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# SUBACUTE EROSIVE ("PEPTIC") ESOPHAGITIS

## Histopathologic Study

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USE OF the terms "peptic" and "regurgitant" in connection with the common, nonspecific form of esophagitis (subacute erosive esophagitis) has implied a definite etiology in a situation which does not seem yet to permit definitive thinking. Determination of the true basic, or primary, cause of subacute erosive esophagitis is particularly important because, to date, therapy directed at the acid-peptic influence has been ineffective in preventing its complications. Esophageal stricture and obstruction continue to be difficult therapeutic problems. The development of deep ulceration and perforation, although rare, is catastrophic. Sudden generalized erosion on occasion still leads to rapid exsanguination, and it is here that more properly directed prophylaxis is especially important. Because current nonsurgical methods for treating esophagitis, largely designed to subdue acid-peptic influence and to discourage transcardial reflux, have been largely impotent in discouraging these complications, it has become the practice in certain centers to treat selected cases by subtotal gastrectomy in an effort to attack gastric secretion more effectively; however, the results at this hospital and elsewhere * have been discouraging.

It has previously been found [1] that the clinical facts do not support the thesis that acid-peptic corrosion is the primary etiologic factor in "peptic" esophagitis. A better understanding of the pathology of the disease may be expected to help in elucidating the importance of regurgitant corrosion. Pathologic investigations have been made almost exclusively on autopsy material, and it has not been possible to interpret these findings without a degree of uncertainty as to the part played by agonal and postmortem autodigestion. The present study was made on transesophagoscopic biopsy specimens.

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* Craighead, C. C., Jr.: Personal communication to the author, 1954.

### MATERIAL AND METHODS

One hundred patients with the esophagoscopic diagnosis of subacute erosive esophagitis were studied. Clinical details regarding the patient group have already been presented.[1] It was pointed out that the gross pathologic changes, as observed esophagoscopically, consist of erosions, hyperemia, and an exudate composed largely of desquamated epithelial cells.

Biopsy specimens were taken of areas which showed these changes in most of the 100 patients, but only tissue which included full mucosal thickness, with the entire muscularis mucosae, was considered adequate for the present study. There were such specimens in the cases of 61 patients, and in 12 of these, satisfactory specimens had been obtained at more than one examination. When serial specimens had been taken, the first was used for study of the incidence of various abnormalities, and the others, only for study of dynamic changes. Hematoxylin and eosin stains were used.

### FINDINGS

*Epithelium.*—There were 36 specimens in which the epithelium was entirely normal. An erosion had been included in 13 specimens. Only one of these erosions extended through the epithelial layer, and here the base of the ulcer consisted of fibrosis and granulation. In the other 12 specimens the stratum germinativum and lower squamous strata were intact. The base of the erosions was composed of squamous cells, which either were normal or showed hyaline degeneration (Fig. 1). There was no inflammatory reaction at the eroded surfaces. The exfoliated epithelial sheet was still attached at one edge in three of the specimens, and

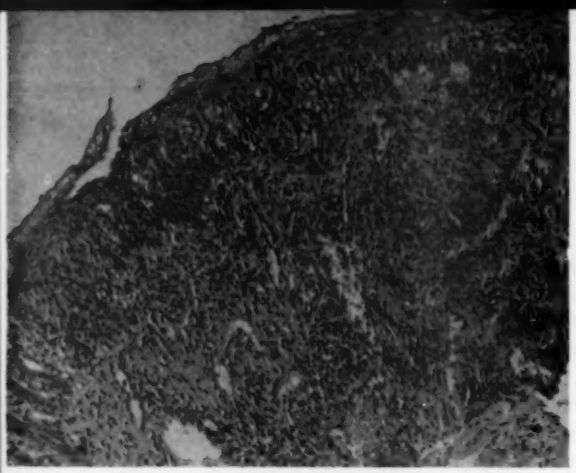


Fig. 1.—Erosion. The surface shows no inflammatory change. The chronic inflammatory infiltrate of the lamina propria mucosae has invaded the stratum germinativum in some areas and has progressed part way through the remaining epithelium, toward the erosion base. Reduced 2/5 from mag. × 110.

the squamous cells composing it were entirely normal, without necrosis or inflammatory infiltration.

The specimens from two patients showed hyperplasia of the stratum germinativum, with growth of the layer, many cells thick, down into the lamina propria mucosae in the form of broad projections (Fig. 2). In only two specimens was there hyperplasia of the more superficial epithelial cells, with significant thickening of the epithelial layer.

Inflammatory infiltration of the epithelial layer from below was found in specimens from 19 patients (Figs. 1 and 3). The concentration of inflammatory cells was never nearly so great in the epithelium as it was in the underlying connective tissue. In most instances small areas of the stratum germinativum had been disrupted by the infiltration. The inflammatory cells had migrated less than halfway through the epithelium in most cases. Except for the stratum germinativum, the individual epithelial cells did not appear to have suffered from the inflammatory infiltration. The infiltrate was composed largely of plasma cells and lymphocytes. Occasionally a few neutrophiles were found. Eosinophiles were regularly found in small numbers, and in one specimen in tremendous numbers, scattered through the epithelium and aggregated in the vicinity of the stratum germinativum.

*Rete Pegs.*—These were abnormal in nine cases: There was notable vascular dilatation and engorgement, with tiny hemorrhages, in three; minute, well-localized abscesses in one; edema, causing enlargement of the pegs, in one, and heavy round-cell infiltrations plugging the pegs in four. It was found in many instances that pegs which were normal extended into epithelium which was diseased. Furthermore, epithelium overlying lamina propria which was severely inflamed often contained rete pegs which were normal. In all instances in which pieces of exfoliated epithelium were included with the specimen, the pegs in the cast-off sheets were normal.

*Lamina Propria Mucosae.*—In all cases this layer was abnormal, and in all by far the greatest part of the disease was found here (Fig. 4). The disease was sometimes rather patchy, small areas of inflammation alternating with small areas of relatively normal

Fig. 2.—Hyperplasia and focal chronic inflammation of stratum germinativum; reduced 2/5 from mag. × 110.

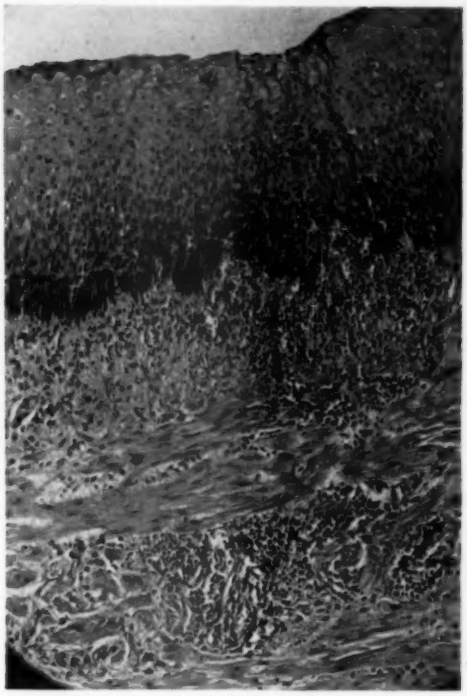


Fig. 3.—Light chronic inflammatory infiltration of the lamina propria mucosae, which has extended into both the base of the epithelium and the muscularis mucosae; reduced 2/5 from mag. × 110.

lamina propria. The three main features were cellular infiltration, fibrosis, and edema.

The cellular exudate was composed largely of lymphocytes and plasma cells, but a few sections showed many eosinophiles. Occasionally large numbers of neutrophiles were found. The infiltration was heavy and diffuse, with some tendency toward poorly defined aggregation. In some cases the entire thickness of the layer was packed with round cells, with upward extension through the stratum germinativum into the epithelium and downward infiltration into the muscularis mucosae. In other cases the exudate was restricted to the lamina propria and took the form of a diffuse infiltration, with scattered areas of cellular aggregation. In general, the maximum amount of inflammation was found to lie closer to the epithelium than to the muscularis mucosae.

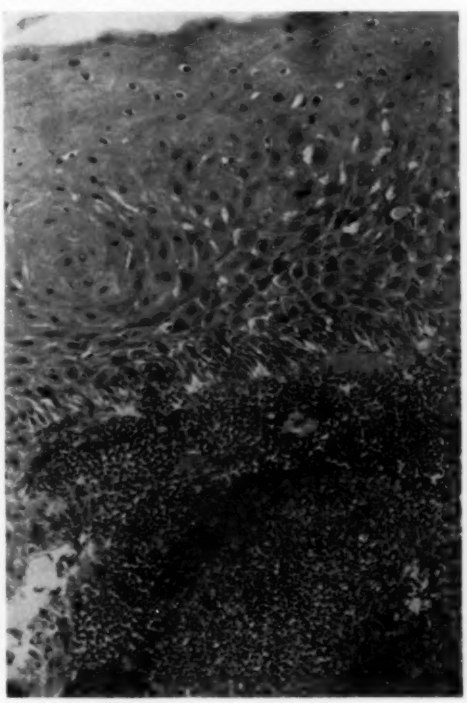
Fibrosis was found in the lamina propria of the specimens from 12 patients, usually in small amounts. There was edema through the connective tissue in six.

*Muscularis Mucosae.*—This layer was considered normal in 41 patients, with regular arrangement of the muscle septa and absence of fibrosis, inflammatory infiltration, atrophy, and fragmentation. In six cases there was prominent edema, separating the muscle bundles. In 14 others the layer was disrupted by areas of fibrosis and heavy accumulations of round cells (Fig. 5). Lymphocytes and plasma cells predominated, but in one specimen there were masses of eosinophiles.

### SERIAL BIOPSIES

Biopsy specimens were taken at successive esophagoscopic examinations of 12 patients, in an effort to add, as much as possible, a dynamic note to what would otherwise have been a purely static evaluation of esophagitis. The patients were at the time under simple treatment for esophagitis; the nature of the

Fig. 4.—Heavy acute and chronic inflammatory infiltrate of the lamina propria mucosae, extending to the stratum germinativum but not invading the epithelium; reduced 2/5 from mag. × 110.



*Results of Serial Biopsies in Twelve Patients*

| Patient | Interval, Wk. | Description of Specimen |
|---|---|---|
| 1 | | Moderate esophagitis |
| | 2 | Little change, more fibrosis |
| | 25 | Less cellular infiltration, less fibrosis |
| 2 | | Moderate esophagitis, many eosinophiles |
| | 2 | Unchanged |
| | 2 | Unchanged |
| 3 | | Mild esophagitis, much edema |
| | 6 | Less edema, more infiltration |
| | 7 | Unchanged |
| 4 | | Severe esophagitis |
| | 2 | Unchanged |
| | 6 | Unchanged |
| 5 | | Moderate esophagitis |
| | 3 | Small areas of acute infiltration |
| 6 | | Severe esophagitis |
| | 11 | Minor improvement |
| 7 | | Moderate esophagitis |
| | 4 | Unchanged |
| 8 | | Moderate esophagitis |
| | 2 | Unchanged |
| | 4 | Unchanged |
| | 3 | Unchanged |
| 9 | | Severe esophagitis |
| | 2 | Unchanged |
| | 4 | Unchanged |
| 10 | | Moderate esophagitis |
| | 12 | More infiltration |
| | 19 | Unchanged, except more edema |
| 11 | | Moderate esophagitis |
| | 8 | Much less infiltration |
| | 3 | More fibrosis and infiltration |
| | 6 | More fibrosis |
| 12 | | Mild esophagitis |
| | 4 | Unchanged |

treatment and the changes in the esophagoscopic picture have previously been described.[1] Thirty-four specimens were taken, over periods of from 3 to 31 weeks.

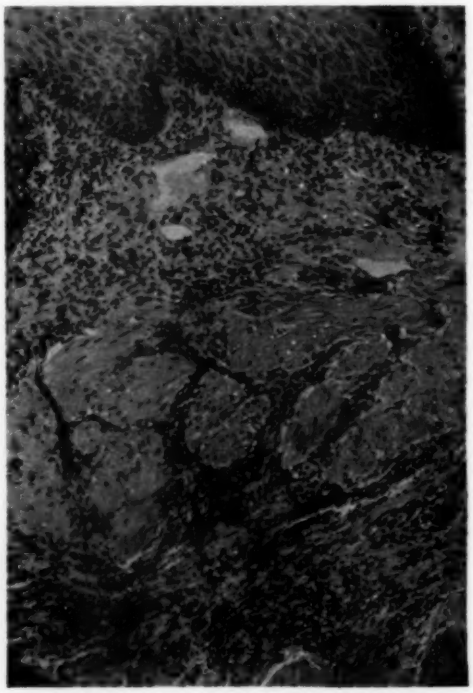
The findings are summarized in the accompanying Table. Detailed comparison of serial specimens from a single patient would presuppose that each biopsy specimen had been taken from the same area of the esophageal wall or that esophagitis is a uniformly diffuse disease, everywhere in the same stage of development. Neither situation obtains, and, therefore, any comparison can only be most general. The important point brought out by the data is that over considerable periods of time the histopathologic pictures showed little quantitative or qualitative change.

## COMMENT

The histopathologic findings are interpreted as demonstrating a primary subepithelial disease, with surface erosion which is merely secondary to the underlying process. The erosions were characterized by absence of inflammation, and, except when the specimens included an erosion, the surface was normal. The picture suggested that the inciting influence had been working beneath the epithelium.

Surface necrosis has been reported in some studies made on autopsy material, but none was found in the present biopsy investigation. Hamperl[2] described epithelial necrosis, although it is important to note that he concluded that erosions are not produced by corrosion downward from the surface, but are caused by exfoliation of the intact superficial layer of epithelium through a stratum of hyaline change. The small areas of necrosis described by Mosher[3] were associated

Fig. 5.—Fibrosis and infiltration of the muscularis mucosae, with little infiltration of the lamina propria mucosae; reduced 2/5 from mag. × 110.



with demonstrable bacterial invasion and minute acute abscesses. Butt and Vinson [4] found widespread "denuding" of the epithelium and, in a few cases, pseudomembrane production, but the specific pathologic activity which had been behind these changes was not made clear.

It was the thesis of Bartels,[5] Hamperl,[2] Neubürger,[6] and others that the normal esophageal mucosa has an efficient mechanism which protects against the digestive action of gastric juice. They postulated that some noxious influence must first weaken the epithelial surface before it will yield to enzymatic corrosion. The nature of the influence has not been identified. Surface mucus has frequently been cited as the protective mechanism but in certain situations which encourage esophagitis a protective mechanism acting only on the surface could not be considered important. Thus, hematogenous poisoning is suggested as one devitalizing influence by the common finding of esophagitis in patients who have died with uremia [4] and hematogenous infection and by the common association with peritonitis, pneumonia, and septicemia.† The Cushing and the Curling phenomena appear to be at work in some instances of esophagitis, although, in so saying, one is probably speaking of mechanisms which are far peripheral to the common final pathway.

Penner and Bernheim [7] and Bloch [8] believed that the devitalizing influence might often be ischemia secondary to shock, especially in esophagitis which follows major surgery. Among Bloch's 20 cases of esophagitis studied at autopsy, there were 11 with the history of a recent operation accompanied by shock. Penner and Bernheim postulated that intramural damage in this situation is the result of arteriolar contraction, leading to a drop in the capillary pressure gradient, hypoxia, loss of capillary integrity, hemorrhage and exudation. At the moment, there are not enough facts to permit implication of arteriovenous shunts in the esophageal wall as the primary cause of significant mucosal hypoxia, devitalization, and esophagitis, similar to their possible role in production of gastric and duodenal ulcers. Nevertheless, the incidence of ulcer in patients with esophagitis is high enough ‡ to encourage search for a common etiology. It may be assumed that in both "peptic" esophagitis and "peptic" ulcer, once tissue devitalization has been produced, the acid-peptic influence may play a secondary role in cleaning away the superficial tissue.

It was Mosher's § contention from autopsy investigation that actual infection within the esophageal wall is the cause of esophagitis. This may come about, he thought, either by the hematogenous route, in patients with bacteremia, or by invasion of the esophageal glands by way of their ducts, from swallowed organisms. He wrote much about the latter mechanism, believing that the glands play a large part in esophageal inflammation. He often found both glands and ducts dilated and surrounded by chronic inflammatory exudate. Spread of inflammation through the lamina propria mucosae and into the muscle layers appeared to him unrestricted, once infection had become established in the glands. Mosher has contributed too many good ideas to esophagology to permit blanket refutation of his theory, but it must be pointed out that most investigators have been unimpressed by the amount of disease concentrated in and about the glands in subacute erosive esophagitis, and that the type of histopathologic disease encountered in this form of esophagitis is not that which can be ascribed to specific bacterial infection. Perhaps Mosher was studying a different kind of esophagitis; certainly, his thoughts would apply to some cases of phlegmonous esophagitis. One notes that Auguste,[13] with tongue in cheek, mentioned the possibility that the presence or absence of septic environs might explain the contradiction created by the proposal that "anti-peptic" operations, which are known to encourage esophagitis in some patients, be employed for the treatment of esophagitis.

† References 3 and 4.

‡ References 1, 4, and 9 through 11.

§ References 3 and 12.

Wangensteen has been one of the few who have not been impressed with esophageal resistance to gastric juice. In 1953 he stated [14]: "Lessons learned . . . have served to indicate how very sensitive the esophageal mucosa is to injury by the digestive juices." He was able to produce severe acute esophagitis in dogs within a few days by obstructing the pylorus and giving intramuscular histamine in yellow wax U. S. P. (beeswax) daily. Selye [15] quickly produced acute hemorrhagic erosions in the esophagus of rats by mere ligation of the pylorus. Furthermore, Wangensteen [14] reported that secretions from the isolated canine gastric pouch caused esophageal damage quickly when run through the esophagus of other dogs, and in the cat the secretions produced esophageal perforation within 20 minutes by mere contact!

It is the histopathologic picture together with the concept that reduction of tissue vitality is required before living tissue may be digested that has made it impossible to accept the hypothesis that the direct cause of "peptic" esophagitis is peptic activity, whatever secondary part it may play. A compelling argument to support the position is that, although "peptic" esophagitis is a rather common accompaniment of "peptic" ulcer and diseases which encourage transcardial reflux of gastric contents, it also is encountered in achlorhydria, following total gastrectomy, and as a sequel of stomach operations specifically designed to reduce gastric secretory activity.[13] The protagonists of the corrosion theory have had to fit this observation into a scheme which had been proposed long before gastric surgery became a factor for consideration.[16] This necessarily had to be done by extending the proposed causative factor from the acid-peptic influence alone to all gastrointestinal secretions which are foreign to the esophagus. Thus, the protagonists themselves demonstrated that bile and pancreatic juice are able to cause esophageal erosions and that there is enough regurgitation of these juices after total gastrectomy and duodenoesophagostomy in the dog to produce esophagitis.[17] Furthermore, it was shown in the dog that development of esophagitis is not altered by total excision of the acid-secreting portion of the stomach.[17] Another point of seemingly irreconcilable confusion is thus added to the faltering theory that acid-peptic influence per se can produce esophagitis and gastroduodenal ulcer: Now small intestine contents, which had meanwhile been proposed as an effective agent in counteracting acid-peptic influence in the treatment of "peptic" disease, are found to be an equally injurious influence.

## SUMMARY AND CONCLUSION

The histopathology of subacute erosive esophagitis is reviewed in the light of biopsy specimens obtained from 61 patients. Evidences of disease were localized largely to the lamina propria mucosae. The epithelial layer itself was normal or merely secondarily diseased, either by inflammatory exudate extending upward from the lamina propria or by noninflammatory superficial exfoliation. Serial biopsy specimens removed at variable intervals from 12 patients showed the histopathologic pictures to be notably static.

It is concluded that histopathologic information does not support the contention that "peptic" esophagitis is caused by the corrosive action of regurgitated gastric juice, although, once mucosal vitality has been impaired, secretory products may help to remove dying tissue.

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# HISTOCHEMICAL STUDIES OF FIBRINOID SUBSTANCES AND OTHER ABNORMAL TISSUE PROTEINS

## I. The Application of Roque's Chromotrope Stain

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FIBRINOID alteration of connective tissue and blood vessels is revealed by routine hematoxylin-eosin stain by the presence of an eosinophilic, highly refractile mass deposited between collagen fibers and within the vessel wall. This definition of fibrinoid is based on superficial microscopic observations and gives no information as to its nature or mode of formation.[1] Substances possessing this tinctorial quality have been observed in a variety of etiologically different diseases. Altshuler and Angevine,[2] after studying different fibrinoids, concluded that the material represented precipitated acid mucopolysaccharides. This broad conclusion does not appear to be entirely justified. Extensive studies [3] on the nature of the fibrinoid in fatal cases of disseminated lupus have demonstrated an evolutionary relationship to deoxyribose nucleoprotein. The character of the fibrinoids in other conditions is not so well known, except that on the basis of general staining properties they are largely protein in nature. The microscopic distinction between fibrin and fibrinoid may be difficult, and the proposition that some types of fibrinoid represent altered fibrin is open to serious consideration.[4]

In an attempt to elucidate the protein complex of fibrinoid or differences in various fibrinoids, the chromotrope 2R-aniline blue procedure was studied. Roque [5] originally developed this method to examine the Mallory bodies of Laennec's cirrhosis. He found the stain to be of value in showing the mixed nature of hyalin, which was not revealed by routine stains. When hyalin fixed both the chromotrope and the aniline blue, he considered this to be a demonstration of more than one protein. This conclusion was confirmed in this laboratory by indirect methods, owing to the limitations of present techniques. The purpose of this paper is to report a series of observations utilizing this staining method, especially as it applies to the analysis of protein substances.

### METHOD

The original method of Roque was found to be entirely adequate. A critical point in the procedure is the maintenance of the dye bath at pH 2.0 to 2.2. Changes in the pH tend to render the stain nonselective, with a loss of clarity of structural detail. Once the staining solution is prepared, it will remain suitable for use after three months' storage in a brown-glass bottle. If the stain is allowed to remain in the staining dish, after repeated use the pH may shift and precipitates may form. When precipitation is noted, the stain should be discarded.

When phosphomolybdic acid was substituted for phosphotungstic acid in the preparation of the aniline blue dye, the end-result was not satisfactory. The blue color seemed to diffuse through the tissue, obscuring chromotrope-positive (red) material. Trichloroacetic acid, another protein precipitant, was tried as a substitute, with equally poor results.

A variety of fixatives were studied, including neutral formaldehyde solution U.S.P. (acetate buffer), 10%, modified Carnoy solution,[3] alcohol, and acetone. Formaldehyde and Carnoy fixation gave the best results, and these substances were used

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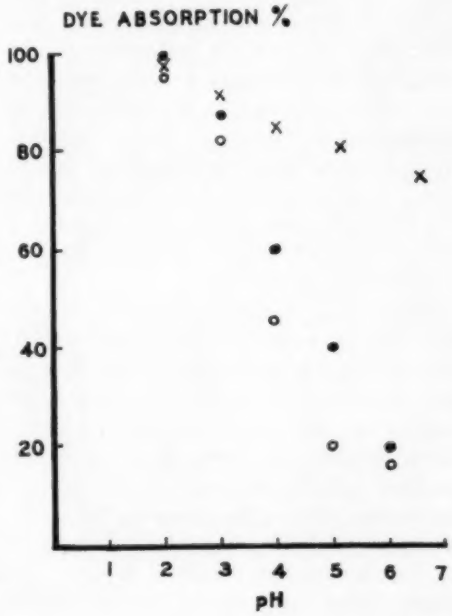
routinely. Fixatives containing mercury salts or procedures utilizing chromates yielded unsatisfactory results. A freeze-dry method produced well-stained tissues. In most instances, irregularity in staining quality of tissue sections was due to inadequate fixation. Formalin-fixed bone subjected to decalcification by inorganic acids could be adequately stained.

The effect of heat on the staining procedure was determined by placing the staining dishes containing tissue sections in the 37 C. or 56 C. incubator for 12 to 16 hours. Maintaining these temperatures did not appreciably alter the final product as compared to the usual method.

### SPECIFICITY OF THE METHOD

Roque [5] studied formalin-fixed histone, glycoprotein, serum gamma globulin, serum albumin, fibrinogen, fibrin, and hemoglobin samples as to their staining properties. Glycoprotein bound the aniline blue, while the other proteins fixed the chromotrope and stained red. From this observation he suggested that the aniline blue in combination with phosphotungstic acid had a special affinity for glycoprotein. Comparing serial sections with chromotrope-aniline blue, periodic acid-Schiff reagent (PAS), and toluidine blue staining procedures, we noted considerable variations. Structures which were PAS-positive were often aniline-blue-negative. Metachromatic tissue components demonstrable by toluidine blue showed most frequently a pale pink-blue after chromotrope-aniline-blue staining but never an intense blue. Collagen fibers which constantly stain dark blue were not affected in the ability to fix this dye after pectinase digestion of the tissues. The enzyme was used over a concentration range from 0.5% to 5.0% at pH 3.6. Mild proteolysis produced by pepsin and trypsin also did not decrease the ability of collagen fibers to bind aniline blue.

With the use of fibrin films, the uptake of the acid dyes, chromotrope 2R, and aniline blue were individually studied at varying pH. At pH 2.0 maximal dye binding was assumed, since dissociation of basic proteins is complete.* It can be seen (Figure) that the affinity of chromotrope for fibrin is extremely sensitive to pH changes while aniline blue binding remains relatively unaltered. Fibrin fixed in formaldehyde (formalin 10%, 12 to 16 hours) showed a decreased affinity for the chromotrope dye as compared with that for the unfixed material (Figure). Fibrin clots fixed by freeze-drying and then cut at 10μ revealed a similar loss of affinity for chromotrope.




Chromotrope 2R (solid circle) and aniline blue (X) absorption by unfixed fibrin. Chromotrope 2R (open circle) absorption by formaldehyde-fixed fibrin. (Modified from Roque.)

### STUDIES ON FIBRINOID AND OTHER ABNORMAL PROTEINS

Formaldehyde- and Carnoy-fixed tissues from fatal cases of scleroderma, disseminated lupus, rheumatic fever, malignant hypertension, and multiple myeloma were studied. Control sections were stained with hematoxylin-eosin, periodic scid-Schiff (PAS), phosphotungstic acid hematoxylin, and silver (Wilder) techniques. Occasional sections were stained by an azocarmine method

* References 6 and 7.

(Mallory-Heidenhain). In several cases the fibrinoid material was observed in serial sections.

The fibrinoid substance noted in lupus, scleroderma, rheumatic fever, and malignant hypertension usually stained red to brilliant red. However, in some instances there was a peripheral margin of light pink or pink-blue material which blended with the central red mass. This was especially true in scleroderma, where, on occasion, pools or lakes of pink-blue material deepen in color at one margin, reaching an intense blue color identical with the coloration of the collagen fibers. Verrucae of acute rheumatic fever were composed of a central core of chromotrope-positive fibrinoid material, which showed lighter gradations of red to light blue as a mantle around the periphery. The fibrinoid in the vessel walls in cases of malignant hypertension stained chromotrope-positive in all cases.

Protein casts in the renal tubules of myeloma kidneys were advantageously studied. Surrounding the glomerulus, lying free in Bowman's space, were chromotrope-positive globules or droplets as distinct structures. These globules were next observed in the proximal tubules as conglomerate masses. The central part of the mass was an amorphous red, while droplets could be seen fusing together at the margins. A faint but definite blue color could be detected at the borders in focal areas. In other proximal tubules and in the distal portions the casts were completely amorphous, staining predominantly red but with blue intermingled. The casts appeared to be the result of seepage of protein-rich fluid from the glomerulus into Bowman's space, followed by a progressive loss of fluid. As the protein droplets are concentrated, they tend to fuse, lose their identity, and become an amorphous, dense cast.

The chromotrope stain has been valuable in demonstrating hyalin droplets in the epithelium of the renal tubules. The majority of these droplets were chromotrope-positive, but it was possible to observe droplets which were pale blue interspersed at random with the red. Amyloid and paramyloid did not fix the chromotrope or aniline blue when stained in the usual manner. Instead, these proteins stained an indeterminate pink-blue. The observation of Roque [5] that the Kimmelstiel-Wilson lesions in the kidney bind aniline blue while the wire-loop renal lesions of lupus are chromotrope-positive was repeatedly confirmed. Hyaline membranes of the lungs, as noted in premature infants, almost always stained blue. In contrast to this finding, hyaline membranes observed in pneumonitis of unknown nature bound the chromotrope dye. Fibrin fibers constantly stained red.

## COMMENT

The original work of Roque [5] has been confirmed and extended. At controlled pH (2.0 to 2.2), the stain is at maximum effectiveness. Theoretically, at this pH basic proteins are completely dissociated and dye binding is maximal. Both chromotrope 2R and aniline blue are acid dyes, but the former is much more sensitive to pH variation. Many workers † have emphasized the importance of pH in staining procedures, since the uptake of dye by a protein depends on the nature of the electrostatic charge. As pH is shifted, swelling of the protein framework may occur, facilitating the dispersion of dye aggregates. Thus, in addition to the reactive protein groups binding dye ions, the protein micellae may separate and allow the penetration of molecular aggregations of dye.‡ In such a case, the fixation of dye by the protein is obviously nonspecific.

The proteins in histological sections may be considered to be in a "solid phase" owing to the process of fixation. This state is in contrast to soluble proteins in unfixed tissues. The exact effect of formaldehyde on proteins remains obscure. One cannot, therefore, equate the staining properties of "solid phase" protein with its soluble phase.[9] Protein in vivo is in dynamic metabolic equilibrium and when fixed may differ signifi-

† References 6 through 8.

‡ References 8 and 10.

cantly in its properties when compared with the isolated protein. While fixed glycoprotein bound the aniline blue dye selectively, this degree of specificity could not be readily applied to fixed tissue proteins. Aniline blue-positive structures were frequently PAS-negative, not metachromatic, and were not altered by pectinase digestion.

Phosphotungstic acid proved to be an essential component of the staining solution to insure binding of aniline blue. Nonmetallic protein precipitants proved to be unsatisfactory in this respect. Tissue fixed with mercuric chloride failed to bind the dye in a clear manner. This result would indicate that the heavy metal interferes with the binding sites of the protein to reduce its affinity for the dye. Only when the phosphotungstic acid is mixed with the aniline blue first is the dye suitably fixed by the tissue. Apparently, a reactive complex is formed between the acid and the dye, which is more easily bound by the protein. This fact may also help explain the relative lack of specificity of this dye in the staining procedure.

With the use of purified human fibrinogen, fibrin films were made by the method of Singer and Morrison.[8] As seen in the accompanying Figure, the binding of chromotrope 2R dye by fibrin is largely dependent on pH when ionic concentration and dye concentration are kept constant. Aniline blue binding was much less sensitive to pH variation, indicating that the net charge of the protein was not important for this dye. Formaldehyde-fixed fibrin did not bind the chromotrope as vigorously as the unfixed protein. This observation is in essential agreement with the findings of Singer and Morrison,[8] who found that formaldehyde fixation of fibrin decreased its affinity for orange G, an acid dye. It appears that formaldehyde decreases the net positive charge of the protein in some unexplained manner. Controlled enzymatic fibrinolysis revealed fixed fibrin to be much more resistant to lysis than the unfixed protein. The implications of this finding and others will be presented in another report.[10] The physical state of the protein assumes primary importance in developing staining procedures.

The continued need for caution in evaluation of staining methods for proteins is strongly emphasized. Before any degree of specificity is assigned to a given method, all the possible variables which might influence the final result must be studied in detail. The chromotrope 2R-aniline blue staining procedure does not appear to possess any special affinity for a specific protein or class of proteins. Since the method is most sensitive at pH 2.0, it may be stated that basic proteins as a group are selectively stained. The usefulness of this method lies in its ability to demonstrate clearly the heterogeneity of abnormal proteins. Changes in the tinctorial quality of a protein substance based on its affinity for the chromotrope dye under reproducible conditions is good evidence that more than one protein or an altered protein is present. The aniline blue staining serves as an excellent contrast to the red chromotrope. The most interesting observations concern the presence of pink or pink-blue substances in the ground substance. This protein complex often was seen to blend smoothly with a more definite staining structure. One may speculate as to whether the indeterminate staining material serves as a precursor for the sharply stained substance.[11] The sensitive chromotrope component of the staining bath thus becomes extremely useful in detecting differences in protein-containing structures. When used with the routine staining procedures, the chromotrope-aniline blue stain becomes a valuable addition to the pathologist's armamentarium in evaluating tissue dynamics.

SUMMARY

A chromotrope-aniline blue staining procedure was studied and found to have little histochemical specificity.

The binding of chromotrope 2R dye is primarily a function of pH.

This procedure is useful as an adjunct to other routine stains in detecting differences in protein substances.

The physical state of the protein is important in determining the degree of dye fixation.

While present methods limit the determination of the exact nature of tissue protein complexes, the chromotrope-aniline blue procedure can point to protein heterogeneity, which other methods fail to demonstrate.

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# HISTOCHEMICAL STUDIES OF FIBRINOID SUBSTANCES AND OTHER ABNORMAL TISSUE PROTEINS

## II. Effect of Fibrinolytic Enzymes

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NEUMANN [1] first called attention to the fact that fibrinoid degeneration of the connective tissue is distinguished by the occurrence of hyaline masses or bands which resemble fibrin in their staining properties. That fibrinoid is derived from fibrin has never been substantiated. Most workers agree, on the basis of the available evidence, that the fibrinoid material of the connective tissue and blood vessels is not developed from fibrin.[2] However, the distinction microscopically between fibrin and fibrinoid based on tinctorial properties may be a difficult procedure. In an attempt to make this differentiation by more direct and precise means, enzymatic histochemical methods were investigated.

Blood contains a substance which facilitates the lysis of clots at normal pH values. Milstone [3] showed this material to be present in the blood as an inactive enzyme precursor, called plasminogen. This precursor, or profibrinolysin, can be activated by a variety of factors. The most powerful of these activators is a protein product of streptococcal metabolism called streptokinase. The active enzyme is called plasmin or fibrinolysin. The plasminogen is found in the euglobulin fraction of serum and has been found to possess proteolytic activity similar to that of trypsin.* It was therefore originally believed that the fibrinolysin activity merely reflected one of the effects of a nonspecific proteolytic enzyme. Recent studies by Cliffton and Cannamela † have discredited this theory by the demonstration that human plasminogen is converted primarily to a fibrinolytic rather than a proteolytic enzyme, as compared with trypsin.

Since the fibrinoid substances are largely protein in nature, the possibility of separation from fibrin by use of a specific fibrinolysin was studied.

### MATERIALS

Human fibrinogen ‡: Fraction I of human plasma, 10 mg. per milliliter, in barbital (Veronal) buffer, pH 7.4.

Thrombin §: Bovine thrombin, commercial, in barbital buffer.

Streptokinase ‖: Streptokinase (100,000 units) -streptodornase (25,000 units) preparation used as a solution containing 10,000 units of streptokinase per milliliter.

Trypsin ¶: Crystalline trypsin, 0.1 mg. per milliliter, in phosphate buffer, pH 8.2.

Plasminogen: Prepared by the method of Downie and Cliffton [6] and reconstituted in barbital buffer, pH 7.4.

### METHODS

Fibrinolytic activity is determined by a modified standard clot lysis test. To two sterile 13 by 100 mm. tubes, 5.0 ml. of plasminogen solution is added. To the first tube, 0.1 ml. of streptokinase solution, and to the second 0.1 ml. of buffer, is added. At the same time, two more tubes are prepared, buffer being substituted for plasminogen, using identical volumes with the first series. This second pair of tubes serves as plasminogen, streptokinase, and

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* References 4 and 5.

† References 6 and 7.

‡ Furnished by courtesy of the Physical Chemistry Laboratories, Harvard University.

§ Purchased as commercial product (Parke, Davis & Company).

‖ Varidase, furnished by courtesy of the Lederle Laboratories Division, American Cyanamid Company.

¶ Purchased from the Worthington Laboratories, Freehold, N. J.

buffer controls. All tubes are placed in a 37 C. water bath for three minutes to activate the enzyme.

After this, 0.3 ml. of fibrinogen solution and then 0.1 ml. of thrombin are added to all tubes. The tubes are stoppered and incubated at 37 C. The time after addition of thrombin required to complete lysis of a solid clot is the lysis time. When this time was 16 hours or more, it was not considered significant. To study better the effect of plasmin on solid clots, the enzyme solution was added three minutes after a firm clot developed in the tubes.[5] Enzyme activity was also studied on fibrin films prepared on glass slides.

The effects of various tissue fixatives on fibrin were studied as to subsequent lysis by enzyme. Solution of formaldehyde U.S.P. (10%, acetate-buffered), Carnoy solution, acetone, Zenker-formol, absolute alcohol, and freeze-drying fixation methods were applied. After one hour of fixation, the fixatives were discarded and the slides gently washed in barbital buffer. Activated enzyme solution was added next and the slides incubated at 37 C. It was found convenient to place an experimental slide with its control in a Petri dish during the course of these studies. Solid clots were carefully removed from the tubes and then subjected to a freeze-drying procedure. This material was vacuum embedded and then sectioned. When the sections were mounted on glass slides, they were treated exactly as the fibrin films. This entire experiment was then repeated, substituting trypsin for the plasmin.

Fibrin films which were fixed and those which were both fixed and subjected to enzyme action were stained with hematoxylin-eosin and chromotrope-aniline blue methods.

Formaldehyde- and Carnoy-fixed tissue sections demonstrating either fibrin or fibrinoid were deparaffinized and brought to water. They were then placed in a Petri dish and covered with 20.0 ml. of a preheated (37 C.) plasmin preparation. Unfixed fibrin films served as controls. Incubation at 37 C. for 14 to 16 hours was carried out. The slides were then washed in barbital buffer, routinely processed, and, finally, stained by the hematoxylin-eosin, phosphotungstic acid hematoxylin, and chromotrope-aniline blue methods. In a separate experiment, trypsin was used in place of plasmin.

## RESULTS

Lysis time was easily established and adjusted to three hours. This was also the time necessary for complete dissolution of the preformed clot. Trypsin proved to be equally as effective as plasmin. The complete lysis of the fibrin films was more rapid than was lysis in test tubes, apparently owing to the smaller amount of substrate present. There was no significant difference between the activity of the plasmin and that of the trypsin on these films.

After fixation, the fibrin clots, which had been clear and transparent, tended to change in color, with little alteration of the gel. Zenker-formol, formaldehyde, and Carnoy fixatives regularly caused the fibrin to become cloudy to turbid with decreased translucent properties, becoming on occasion almost opalescent. Alcohol, acetone, and freeze-drying appeared to produce less physical change in the fibrin, but in each case there was a definite turbidity throughout the clot. However, there was no change in the firmness of the clot.

Fibrin fixed by any of the methods mentioned failed to be specifically lysed by the fibrinolytic enzyme system. Increase in enzyme concentration, as described by Fletcher,[5] to approximately 20 times the standard lysis time also proved to be ineffective. Trypsin actively digested the fixed material and, when increased in concentration, produced more rapid proteolysis. At a concentration of 10 mg. per milliliter, trypsin gave the most consistent fibrinolysis.

The fixed fibrin films before and after exposure to the plasmin solution stained red with the hematoxylin-eosin and chromotrope-aniline blue methods.

As would be expected from the in vitro results, the tissue sections were not acted upon by fibrinolysin. Prolonged incubation produced artifactual removal of fibrin. Carnoy-fixed tissues were easily digested by trypsin, the speed depending on the concentration of enzyme used. Tissues fixed by freeze-drying and acetone were also susceptible to tryptic digestion. By utilizing different trypsin concentrations under controlled conditions, differences in the proteolysis of various fibrinoid substances became apparent. Preliminary observations suggest that the fibrinoid material observed in malignant hypertension is easily digested by low concentrations of trypsin, while at similar concentrations the fibrinoid material ob-

served in scleroderma is remarkably resistant to the enzyme. This approach to the nature of the protein moiety of the fibrinoid substances is under investigation.

## COMMENT

The alteration of proteins by the process of fixation hampers the development of histochemical methods. Previous studies showed that formaldehyde decreased the ability of fibrin to fix certain acid dyes.[9] This investigation has demonstrated that fixation effectively blocks the enzyme receptor sites on the fibrin molecule for fibrinolysin. Usually this was associated with a physical alteration in the optical clarity of the clot but did not affect the consistency of the clot. The staining properties of the fibrin produced in vitro closely resembled the fibrin in tissue sections similarly stained. Thus, the microscopist who utilizes the conventional tinctorial criteria for the identification of fibrin studies a protein which has been considerably changed from its native state. This does not invalidate the present criteria but merely cautions the interpretations based on them. The problems as to the relationship of fibrin and the fibrinoid substances continue to be open for further research. The method of controlled protein digestion by purified proteolytic enzymes under optimal conditions appears to be a step in the direction toward the clarification of this problem.[10]

## SUMMARY

Fibrin subjected to fixation by routine methods is resistant to lysis by specific fibrinolytic enzymes.

Trypsin effectively digests fibrin previously fixed.

Carnoy-fixed tissues are susceptible to tryptic digestion. The concept of differential proteolysis based on varying enzyme concentrations is mentioned in the analysis of fibrinoid substances.

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# EFFECT OF VITAMIN $B_{12}$ ON SELENIUM POISONING IN THE RAT

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SELENIUM poisoning of livestock is a problem in certain areas of this country. Several attempts have been made to find some agent to counteract this injury. Moxon and associates [1] found that small amounts of arsenic would reduce the symptoms of poisoning in steers. Lewis and associates [2] and Rosenfeld and Beath [3] concluded from their studies that dietary protein and protein supplements modified the toxicity resulting from selenium compounds. Dubois and associates [4] have shown that glutathione will decrease the lethal effects of acute selenium poisoning. This suggests that selenium may interfere with some sulfhydryl-enzyme system. In a previous study it was shown that selenium inhibited the maturation of erythrocytes in the white Pekin duck and that this effect was inhibited by cysteine.[5] Our observations on the effect of vitamin $B_{12}$ on selenium poisoning in the rat are reported.

## METHODS AND MATERIAL

The rats were males of the Sprague-Dawley strain, weighing approximately 200 gm. each. They were kept in small groups and given water and food ad libitum. The basic ration was Purina chow. These pellets were pulverized, and distilled water containing 0.1 mg. of sodium selenite per milliliter was added, in a concentration of 0.05 mg. of selenium per gram of food. After this food was thoroughly mixed, it was made into small biscuits and dried slowly in an oven. The vitamin $B_{12}$* was given subcutaneously in doses of 200 $\gamma$. This amount of the vitamin was selected arbitrarily. Injections were given daily except on Sundays and holidays. The rats were weighed at weekly intervals. All animals were killed, and the liver was removed immediately. Sections were taken, fixed in 4% solution of formaldehyde U. S. P., and prepared in paraffin sections for histological study. The sections were stained routinely with hematoxylin and phloxine. The remaining liver tissue was placed immediately in a deep freeze, at approximately —20 C. The vitamin $B_{12}$ content of the liver was determined according to the method of Skeggs and associates,[6] which was used by one of us (J. R. C.) in a previous study.[7] The vitamin $B_{12}$ content is shown as Lactobacillus leichmannii activity. The criterion of bacterial growth was acid production as determined by titration, using 0.1 N sodium hydroxide.

## EXPERIMENTAL RESULTS

Seven rats (Group A) were fed the standard ration to which was added selenium and were given subcutaneously 200 $\gamma$ of vitamin $B_{12}$ daily for 33 days. Seven rats (Group B) were fed the standard ration to which was added selenium for a similar period. Six rats (Group C) were fed the standard ration and were given 200 $\gamma$ of vitamin $B_{12}$ subcutaneously for six days. Three rats (Group D) were fed the standard ration for 33 days. Twelve of these rats were killed on the 33d experimental day, and the vitamin $B_{12}$ content of the livers was determined. The results are shown in Table 1. From this experiment it may be seen that rats given a small amount of selenium in their ration have a much larger amount of vitamin $B_{12}$ in their livers than do the control animals fed the same ration. The remaining rats in Groups A, B, and C were continued on the same routine and were killed on the 48th experimental day. The vitamin $B_{12}$ content of the livers is shown in Table 1. The amount

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* The vitamin $B_{12}$ was supplied by Merck & Company, Inc., through the courtesy of A. W. Veasey.

TABLE 1.—*Influence of Dietary Selenium on Vitamin $B_{12}$ Content in Rat Liver*

| Diet | Length of Experiment 33 Days: Rat No. | Length of Experiment 33 Days: Vit. $B_{12}$, γ | Length of Experiment 48 Days: Rat No. | Length of Experiment 48 Days: Vit. $B_{12}$, γ |
|---|---|---|---|---|
| Group A | | | | |
| Standard ration, selenium, and $B_{12}$ * | 1 | 461 | 1 | 505 |
| | 2 | 343 | 2 | 420 |
| | 3 | 499 | 3 | 431 |
| | .. | ... | 4 | 458 |
| Average | .. | 434.3 | .. | 453.5 |
| Group B | | | | |
| Standard ration and selenium | 1 | 214 | 1 | 199 |
| | 2 | 305 | 2 | 264 |
| | 3 | 263 | 3 | 247 |
| | .. | ... | 4 | 279 |
| Average | .. | 260.6 | .. | 247.2 |
| Group C | | | | |
| Standard ration and $B_{12}$ | 1 † | 236 | 1 ‡ | 354 |
| | 2 | 460 | 2 | 512 |
| | 3 | 616 | 3 | 824 |
| Average | .. | 437.3 | .. | 896.6 |
| Group D | | | | |
| Standard ration | 1 | 159 | .. | ... |
| | 2 | 143 | .. | ... |
| | 3 | 107 | .. | ... |
| Average | .. | 136.3 | .. | ... |

* 200 γ given subcutaneously daily.

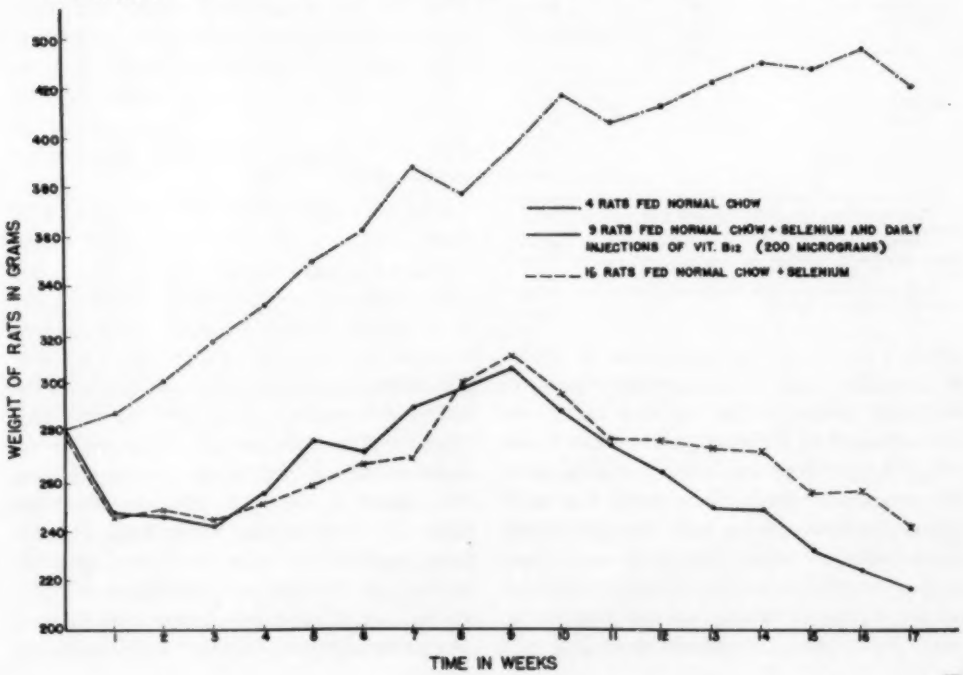
† Four daily subcutaneous injections of 200 γ of vitamin $B_{12}$ were given before these three rats were killed, 24 hours after the last injection.

‡ Six daily subcutaneous injections of 200 γ of vitamin $B_{12}$ were given before these three rats were killed, 24 hours after the last injection.

of vitamin $B_{12}$ in the livers of the rats killed on the 48th experimental day is essentially the same as that found in the animals killed after the 33d day.

A second experiment was set up with 28 rats, averaging 280 gm. each. The animals were separated into three groups and observed for a period of 17 weeks. Four rats, Group X, were fed the standard ration; 15 rats, Group Y, were fed the standard ration with the addition of selenium, and 9 rats, Group Z, were fed the same ration as Group Y with the addition of 200 γ of vitamin $B_{12}$ given subcutaneously each day. The weight curves for these three groups of rats are shown in Figure 1. All the animals receiving the selenium failed to gain, as compared with the controls. There was essentially no difference in the weights of the rats fed selenium and the weights of those fed selenium and given vitamin $B_{12}$ during the first 11 weeks of the experiment. After that time the rats given the vitamin $B_{12}$ weighed less than those given the same ration containing selenium.

Fig. 1.—Weight curves of rats fed a ration containing selenium with and without a supplement of 200 γ of vitamin $B_{12}$ for 17 weeks. Note the decrease in weight as compared with that of the controls.


WEIGHT OF RATS IN GRAMS
TIME IN WEEKS
4 RATS FED NORMAL CHOW
9 RATS FED NORMAL CHOW + SELENIUM AND DAILY INJECTIONS OF VIT. B12 (200 MICROGRAMS)
15 RATS FED NORMAL CHOW + SELENIUM

After 17 weeks four rats in Group X, six in Group Y, and four in Group Z were killed. No significant macroscopic variations were observed other than the loss of weight in Groups Y and Z as compared with Group X. Also, the hair of the rats in Groups Y and Z was yellowish in color, as compared with that of the controls. Histologically, the livers from the 10 rats in Groups Y and Z showed some acute degeneration of the hepatic cells. The foci of degeneration varied in size and extent in the various rats; they were located in different parts of the lobule (Fig. 3*A*). There was some proliferation of the reticuloendothelial cells, and a few small groups of dark-staining cells, consistent with extramedullary blood formation, were found throughout the liver. No variation was noted in the degree of damage to the liver in the rats given selenium and vitamin $B_{12}$ and those getting only selenium. No pathologic changes were observed in the rats fed the standard ration. The vitamin $B_{12}$ content of the livers from these three groups of rats is given in Table 2.

TABLE 2.—*Influence of Dietary Selenium on Vitamin $B_{12}$ Content in Rat Liver*

| | Length of Experiment | | | |
|---|---|---|---|---|
| | 17 Weeks | | 27 Days | |
| Diet | Rat No. | Vit. $B_{12}$, γ | Rat No. | Vit. $B_{12}$, γ |
| Group X | | | Group H * | |
| Standard ration | 1 | 220 | 1 | 41.0 |
| | 2 | 173 | 2 | 78.0 |
| | 3 | 73 | 3 | 53.0 |
| | 4 | 145 | 4 | 57.25 |
| | | | 5 | 63.0 |
| Average | | 155.2 | | |
| | | | Average | 58.45 |
| Group Y | | | | |
| Standard ration and selenium | 1 | 164.0 | | |
| | 2 | 85.0 | | |
| | 3 | 121.0 | | |
| | 4 | 163.7 | | |
| | 5 | 123.6 | | |
| | 6 | 98.0 | | |
| Average | | 125.9 | | |
| Group Z | | | Group Z † | |
| Standard ration, selenium, and $B_{12}$ ‡ | 1 | 422 | 1 | 248.4 |
| | 2 | 380 | 2 | 290.8 |
| | 3 | 251.2 | | |
| | 4 | 340.5 | Average | 269.6 |
| Average | | 348.4 | | |
| | | | Group M * | |
| Standard ration and $B_{12}$ ‡ | | | 1 | 175.2 |
| | | | 2 | 102.0 |
| | | | 3 | 116.7 |
| | | | 4 | 221.7 |
| | | | Average | 153.9 |

* The five rats in Group H and the four in Group M were formerly in Group Y.

† These two rats were formerly in Group Z. One rat from this group died on the 26th day and two on the 27th day.

‡ 200 γ of vitamin $B_{12}$ given subcutaneously daily to rats in Groups M and Z.

To determine whether the loss of weight of the rats in Group Y, given the ration with selenium, was the result of a process that was reversible, the nine remaining animals were divided into two Groups, H and M. The ration containing selenium was discontinued, and the standard ration was given to both groups. One of these groups (Group M) was also given subcutaneously 200 γ of vitamin $B_{12}$ daily for 27 days. The five rats in Group Z were continued on the standard ration containing the selenium supplemented with vitamin $B_{12}$. The weight curves of these three groups of rats are shown in Figure 2. All rats previously fed the standard ration with the addition of selenium rapidly gained in weight when fed only the standard ration. Twenty-six days later one rat in Group Z died, and on the 27th day two more rats in this group were found dead. The remaining two animals were then killed. The vitamin $B_{12}$ content of the livers is given in Table 2. The two rats in Group Z showed extensive acute degenerative changes in the liver (Fig. 3*B*), similar to, but more extensive than, those present in a similar group of rats killed 27 days earlier. A large amount of fluid, resembling a transudate, was present in the serous cavities.

The livers of the rats fed selenium for 17 weeks and then changed to standard ration (Group H) showed regeneration with some fibrosis throughout the liver. Small masses of brownish-yellow pigment were present in some of these areas of scarring (Fig. 3*C*). Few histological changes were present in the livers of the rats (Group M) fed the selenium for 17 weeks and then changed to the standard ration and given the supplement of vitamin $B_{12}$ for 27 days preceding the time they were killed. A few focal areas of fatty degeneration were present (Fig. 3*D*). Fibrosis in the liver was conspicuous by its absence in this group of four rats (Group M) previously fed selenium for 17 weeks and

then changed to standard ration and given 200 γ of vitamin $B_{12}$ for 27 days. No fluid was present in the serous cavities of any of the rats (Groups H and M) previously fed the ration containing selenium and then changed to the standard ration with or without the supplement of vitamin $B_{12}$.

COMMENT

Rosenfeld and Beath [11] have pointed out that sheep fed a ration containing selenium will lose weight because the food intake is reduced. The symptoms of intoxication gradually decreased and the appetite of their sheep increased after the food containing the selenium was discontinued. A similar mechanism may have occurred in these rats (Fig. 1) to account for their loss in weight. It is of interest to note in this experiment that the weight loss was slightly more in the rats given vitamin $B_{12}$ than it was in the group not receiving this supplement. Since the number of animals used in each experiment was small, this variation may not be significant. All rats fed a ration containing selenium rapidly regained their weight when the selenium was discontinued (Fig. 2).

These studies show that when selenium, in a small amount, is included in the ration for a period of only 33 days, the vitamin $B_{12}$ content of the liver is greater (260.6 γ per gram—Group B) than it is in the liver of rats receiving the same ration without the addition of selenium (136.3 γ per gram—Group D). However, when the same amount of selenium is fed to rats over a period of 17 weeks, the vitamin $B_{12}$ content of the liver (125.9 γ per gram—Group Y) decreases. This observation would suggest that the amount of vitamin $B_{12}$ retained in the liver may be related to the severity of the hepatic injury produced by the selenium. A similar relation occurred in the vitamin $B_{12}$ content of the liver in the rats fed the

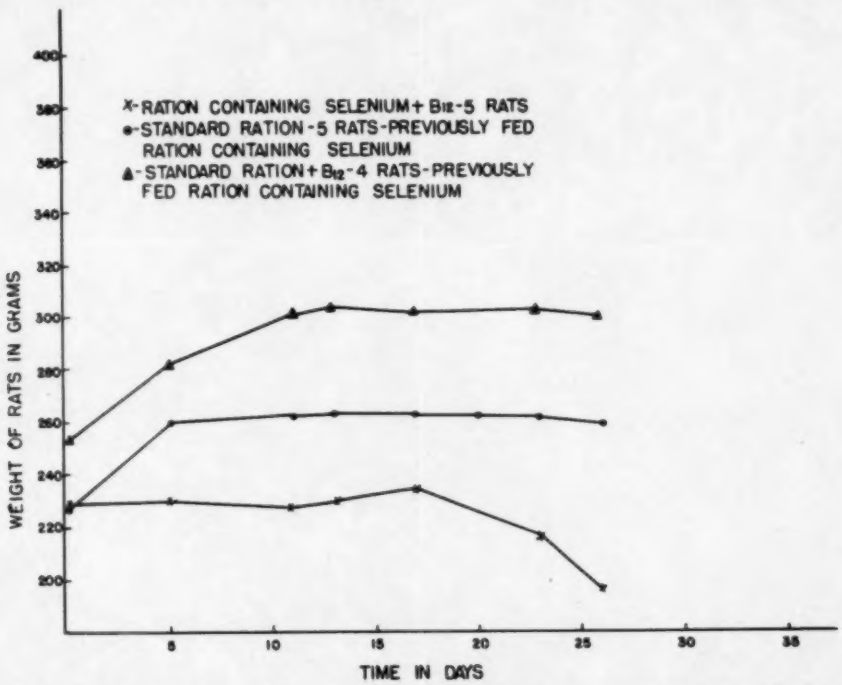



Fig. 2.—Rats fed the standard ration with selenium and given the supplement of vitamin $B_{12}$ continued to lose weight for an additional 27 days. All rats fed the standard ration with selenium for 17 weeks rapidly gained in weight when the selenium was discontinued. Those receiving a supplement of vitamin $B_{12}$ gained more than the group not receiving this additional vitamin.

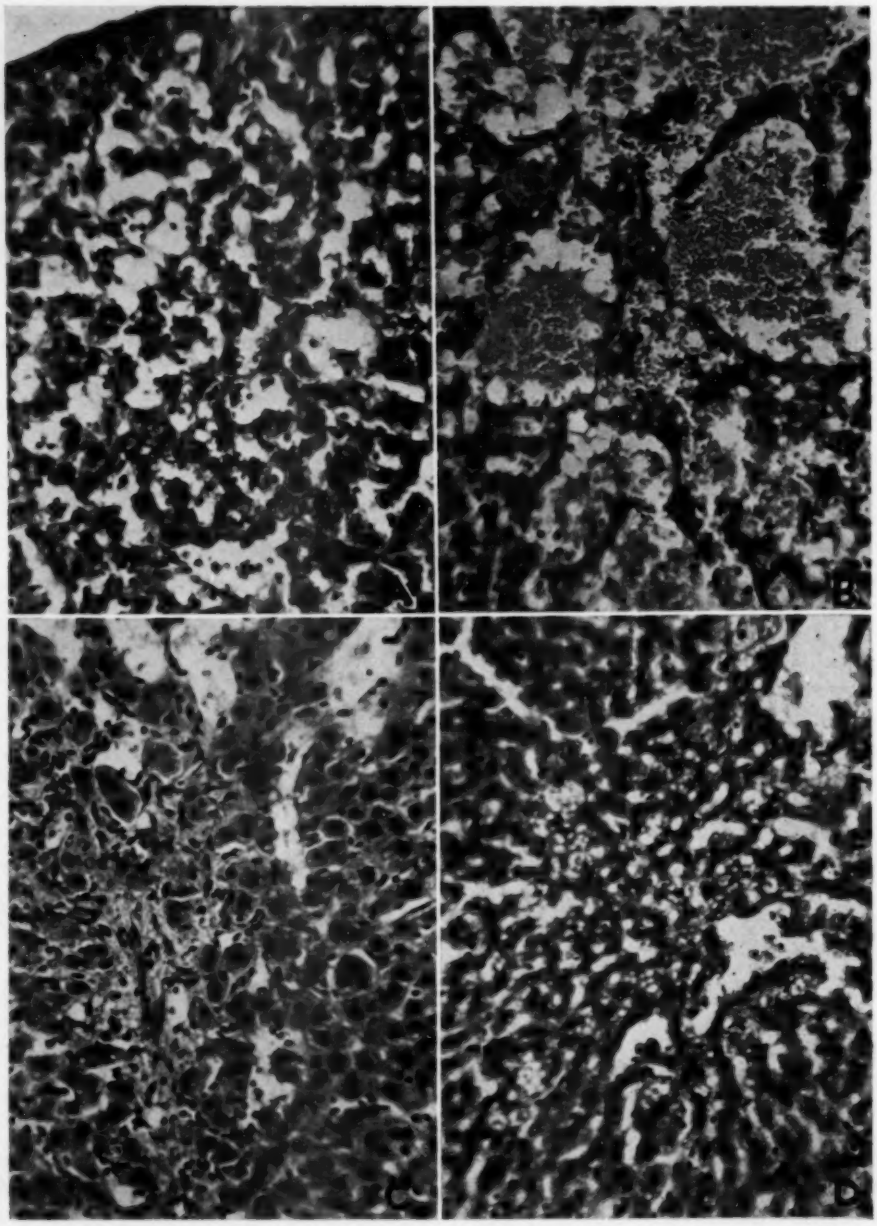


Fig. 3.—*A*, focal areas in which the hepatic cells are degenerated and the sinusoids are dilated, apparently as a compensatory process. Rats were fed the standard ration with selenium for 17 weeks with a daily supplement of 200 γ of vitamin $B_{12}$.

*B*, a similar, but more extensive, lesion in the liver of a rat continued on the same regimen as the rat in *A* for 27 additional days.

*C*, focal areas of scarring are present in the liver of a rat fed the ration containing selenium for 17 weeks and then changed to the standard ration for 27 days.

*D*, focal areas of fatty metamorphosis are present in the liver of a rat fed the ration containing selenium for 17 weeks and then changed to the standard ration supplemented with 200 γ of vitamin $B_{12}$ for 27 days. Fibrosis as observed in the livers of those rats not receiving this supplement is not present in the livers of these rats.

Hematoxylin-phloxine stain; × 180.

ration with the selenium supplement with vitamin $B_{12}$. After 48 days there was 453.5 γ per gram of vitamin $B_{12}$ in the liver of the rats fed a ration with selenium and given a supplement of vitamin $B_{12}$ (Group A), while the vitamin $B_{12}$ content of the liver was only 348.4 γ per gram when the animals were similarly treated for 17 weeks (Group Z).

The livers of rats injured by selenium and then permitted to regenerate for 27 days after the withdrawal of the selenium showed a marked decrease in the content of vitamin $B_{12}$ (58.45 γ per gram—Group H) as compared with that in the control (136.3 γ per gram—Group D). Rats given the ration containing the selenium over a period of 17 weeks and then given the same ration without selenium for 27 days with a 200 γ daily supplement of vitamin $B_{12}$ also showed a much smaller amount of vitamin $B_{12}$ within the liver (153.9 γ per gram—Group M) than did normal rats fed the same ration with an equal supplement of vitamin $B_{12}$ daily for 33 days (437.3 γ per gram—Group C). This latter observation would support the opinion that regenerating hepatic cells either utilize a greater quantity of vitamin $B_{12}$ or are unable to store this vitamin like the normal hepatic cells.

There appears to be no difference in the histologic changes that occur in the livers of rats fed a ration containing selenium with a supplement of vitamin $B_{12}$ and in those without a supplement as used in these experiments. In the rats given selenium there occur focal areas of degeneration of hepatic cells, with resulting cystic areas filled with red blood cells. The process of repair in the liver of rats receiving selenium, however, appears to be influenced by the supplement of vitamin $B_{12}$. There is little fibrosis in the livers of rats receiving large subcutaneous injections of vitamin $B_{12}$ as compared with that in rats not receiving this vitamin as shown in this experiment. It is true that this suggestion is based upon few experimental animals and deserves further study; however, other investigators have linked vitamin $B_{12}$ to nucleoprotein metabolism and liver repair.[8] This fibrous tissue in the liver of rats apparently does not represent true proliferation of fibrous tissue but may represent only a collapse of the hepatic stroma. It would therefore appear that vitamin $B_{12}$ is valuable in the process of regeneration of hepatic cells following injury produced by selenium. In this connection, it is of interest to note that Dumm and Ralli [9] found that "rats receiving vitamin $B_{12}$ showed striking evidences of cellular regeneration" following partial hepatectomy. It has been suggested that this vitamin in large doses may be valuable in the treatment of any case with severe acute liver damage.[10]

The vitamin A and C contents of the liver are decreased in fatal cases of selenium poisoning.† It has been said that this is the result either of insufficient storage or of depletion during selenium intoxication.[11] It would be of interest to know whether the vitamin A and C contents of the liver are increased when the degree of hepatic cell injury is minimal, as we have observed with vitamin $B_{12}$ in experiments carried on for 33 and 48 days. We have nothing to suggest as to why there is a greater amount of vitamin $B_{12}$ in the selenium-treated rats than in the controls at this short interval, whereas the reverse is true in rats observed for 17 weeks. We have no histological studies on rats fed selenium for 48 days; however, from our other histological studies on selenium-fed rats it would seem most likely that few morphological changes would be present in the liver when rats are fed the amount of selenium used in this experiment for only 48 days.

## SUMMARY AND CONCLUSIONS

Rats given a small amount of selenium in their ration for short intervals, 33 and 48 days, have a larger amount of vitamin $B_{12}$ in their livers than have the controls fed the same ration.

The daily subcutaneous injection of 200 γ of vitamin $B_{12}$ in adult rats apparently does not influence the hepatic necrosis that results

† References 11 and 12.

from the ingestion of a small amount of sodium selenite over a period of 17 weeks. Vitamin $B_{12}$ does influence, however, the process of hepatic repair following injury resulting from selenium.

The vitamin $B_{12}$ content of the liver is decreased in rats with severe hepatic necrosis resulting from selenium intoxication.

The vitamin $B_{12}$ content of the liver of rats undergoing regeneration following selenium poisoning is low. It is suggested that regenerating hepatic cells either utilize more vitamin $B_{12}$ than do normal hepatic cells or that they fail to store vitamin $B_{12}$ in the same manner in which normal hepatic cells do.

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# EXPERIMENTAL THYROID METAPLASIA IN THE RAT

**Preliminary Report**

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INCIDENTAL mention of metaplastic changes in the thyroid gland of the rat induced by vitamin A deficiency has appeared in the literature (McCullough and Dalldorf [1]; Hume and others [2]). However, only cursory demonstrations of these frequently prominent manifestations have been presented. Moreover, the origin and significance of such deficiency lesions have escaped notice.

In the present report, attention is directed to the high incidence in rats subjected to a rapid depletion of vitamin A of squamous cell metaplasia involving the specific area of the thyroid gland known as the "ultimobranchial body." Since such sites of metaplasia or certain remnants (cells) derived from them during their repair may persist indefinitely, a recognition of their source and potentialities for aberrant growth is important.

According to Karsner,[3] certain tumors with the tendency to undergo malignant change (struma postbranchialis of Getsowa; parastrumas) have been attributed to this ultimobranchial tissue component in the human thyroid gland. An associated metaplasia, however, is not mentioned. Nevertheless, it is possible that neoplasms of more than one kind may arise from areas of spontaneous metaplasia in the human thyroid (Jaffe [4]). Metaplasias induced by vitamin A deficiency already have proved useful in preliminary investigations dealing with experimental thyroid tumorigenesis in rats (Van Dyke [5]). Such experimental lesions also may have added significance generally, as a "tool," in the study of particular types of neoplasia.

Spontaneous thyroid metaplasia has been observed occasionally in many common mammals, as well is in man. In 1945, I reviewed this subject and pointed out numerous examples of cyst formation in the thyroid glands of presumably normal young sheep. These cysts were composed largely of hyperplastic stratified squamous epithelium and, because of their position and relationships, were assigned to the ultimobranchial tissue component of this endocrine organ. It will be recalled that this structure arises from the caudal end of the embryonic pharynx (entoderm) and usually comes to lie within each lateral lobe of the growing thyroid gland. However, in sheep, unlike many other mammals, it persists typically as a cystic manifestation of metaplasia. This may reflect a deficiency of vitamin A, for the sheep is a grazing, or pasture, animal. Depending, presumably, upon the state of activity of the thyroid gland, the epithelium of these cysts can be the source of peculiar new growths, which may resemble typical thyroid parenchyma or more or less solid adenoma-like nodules. These growths may originate from either the basal (germinative) or from the suprabasal (degenerating) cells in this stratified epithelium (Van Dyke [6]).

In the rat this ultimobranchial tissue becomes indistinguishably transformed into thyroid-like tissue during embryonic development. At birth, it usually cannot be distinguished as a separate entity from any neighboring areas of thyroid tissue (Rogers [7]). According to some investigators, a similar situation holds for the development of the human thyroid gland (Weller [8]; Norris [9]).

In view of this apparent similarity to the embryology of the human thyroid gland, the

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present experiment is an attempt to clarify some of the factors that may influence this ultimobranchial tissue component in very young rats. It was designed not only to demonstrate, if possible, the source of metaplastic lesions within this gland resulting from a deficiency of vitamin A but also to determine their character during periods of growth and repair. It is part of a comprehensive study dealing with metaplasia as an associated agent in the evolution of certain forms of neoplasia, especially in the thyroid gland.

## MATERIAL AND METHODS

A total of 90 female albino rats have been utilized in this study. Sixty of these were Sprague-Dawley animals 22 to 28 days of age. These were divided into four experimental groups of 15 animals each and maintained on a vitamin A-deficient diet for 10 to 40 days (Nutritional Biochemicals Corporation). Twenty Sprague-Dawley rats, in four groups in 5 each, served as normal controls (Groups I to IV, Table).

Ten of the animals in each of the experimental groups were killed at 10-day increments, beginning with the 10th day of the experiment (total of 40 rats). At the same time (at 10-day increments), the five experimental animals remaining in each group were placed on a diet adequate in vitamin A (Purina Dog Chow). One member in each of the four experimental groups was killed on the 2d, 4th, 6th, 8th, and 10th day after the start of the beneficial diet (total of 20 rats). This reparative diet was fortified by a supplement of carotene (Eastman Kodak Company 20 γ per rat per day) mixed with the Dog Chow. Control rats were examined at 10-day intervals through the 40th day.

Ten additional rats (Wistar strain, 30 days of age) were fed the vitamin A-deficient diet for an optimum period of 35 days. After this, they were maintained on the reparative diet and killed after one month. All experimental animals were fed an initial dose of 3 cc. of cod liver oil during the first day of the restorative period.

A few rats died during the course of the experiment and were replaced. All thyroid glands (with attached tracheas) were fixed in Zenker's fluid, embedded in paraffin, and sectioned in the frontal plane at 7 μ. Complete serial sections, stained largely with hematoxylin and eosin, were studied. The results are indicated in the accompanying Table.

## RESULTS AND OBSERVATIONS

The control thyroid glands showed the marked histological variation typical of the normal human thyroid gland. However, metaplasia in the form of small cysts lined by stratified squamous epithelium occurred in or near the center of the thyroid gland in five of these rats (25%). On the basis of previous studies, the position of the cysts in each thyroid lobe has been interpreted as indicating an origin from ultimobranchial tissue. Most of these cysts were encountered in the youngest age groups and may indicate a relative deficiency of vitamin A in certain stock rats (Table). A varying degree of hyperplasia characterized the thyroid gland in these young animals.

Thyroid glands from rats maintained on a vitamin A-deficient diet frequently showed considerable follicular hyperplasia during the first few weeks of the experiment. This was generally followed by degeneration or atrophy of the parenchyma usually after the 20th day of the experimental period (Fig. 1).

*Thyroid Metaplasia in Young Control and Vitamin A-Deficient Sprague-Dawley Rats During Experimental Period of Ten to Forty Days; Repair of Metaplasia in Twenty Rats Following Acquisition of Vitamin A*

| Group | Days | No. of Controls | No. with Metaplasia in Controls | No. of Experimental Rats | Metaplasia in Experimental Rats, No. | Degree * | Repair of Lesions After Carotene, Day | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 2 | 4 | 6 | 8 | 10 |
| I | 10 | 5 | 2 | 10 | 6 | + | M † | R ‡ | R | 0 § | 0 |
| II | 20 | 5 | 2 | 10 | 10 | ++ | R | R | R | R | 0 |
| III | 30 | 5 | 0 | 10 | 10 | +++ | M | R | R | R | R |
| IV | 40 | 5 | 1 | 10 | 10 | ++++ | M | R | R | R | R |
| | Totals | 20 | 5 (25%) | 40 | 36 (90%) | | 1 | 4 | 4 | 3 | 2 |

* + to ++++ indicates extent of metaplasia.
† M, metaplasia but no repair.
‡ R, repair of metaplasia.
§ 0, no metaplastic lesion.

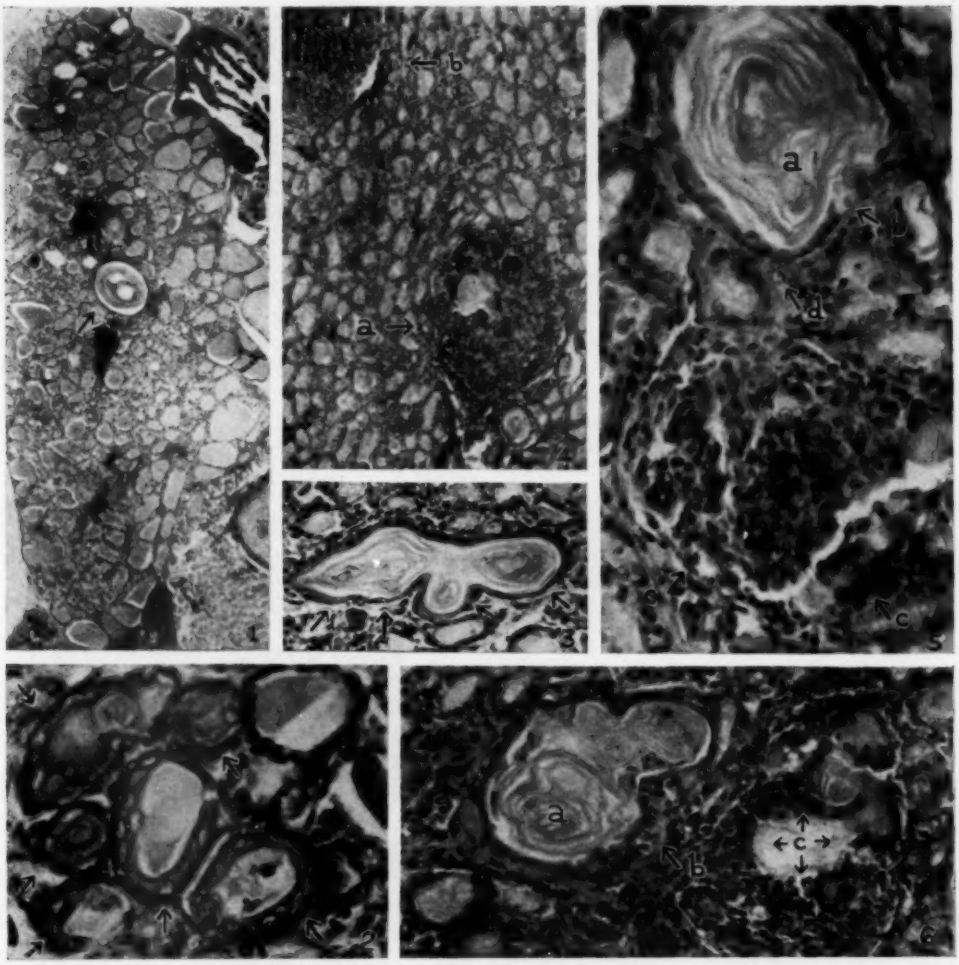



Fig. 1.—Epithelial cyst resulting from metaplasia in thyroid of rat fed vitamin A-deficient diet for 30 days. Note its central position (arrow) within the surrounding partial atrophic thyroid parenchyma. Hematoxylin and eosin; reduced 1/5 from mag. × 25.

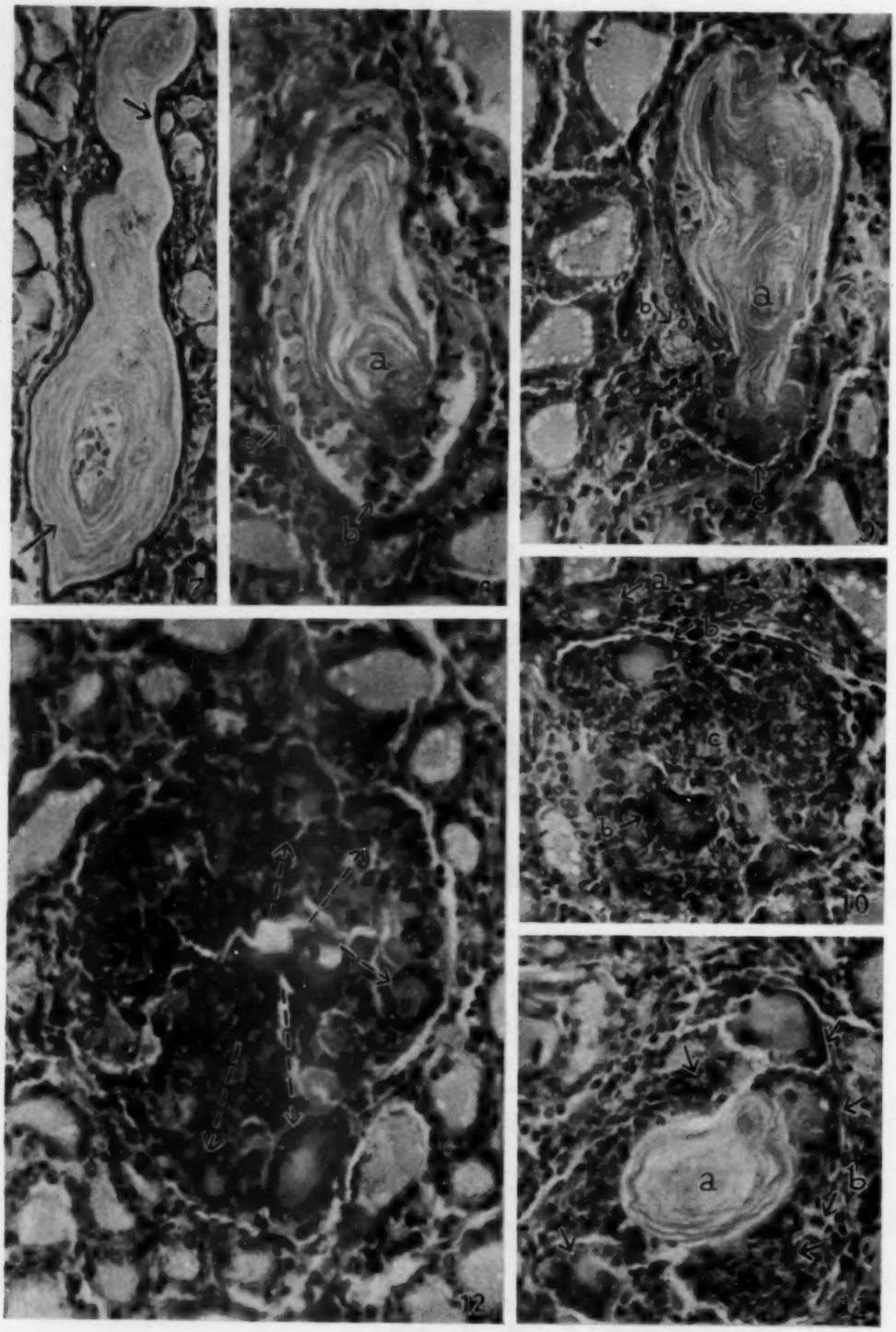
Fig. 2.—Very early squamous cell metaplasia in six separate thyroid-like vesicles (Group I); hematoxylin and eosin; reduced slightly from mag. × 250.

Fig. 3.—Early coalition of apparently four vesicles (arrows) undergoing metaplasia to form an irregularly shaped cyst (Group II). Note the contained individual masses of desquamated cells. Hematoxylin and eosin; reduced slightly from mag. × 80.

Fig. 4.—Metaplastic lesion in process of repair (at *a*) following acquisition of carotene for eight days (Group III). Note relation of this densely staining cell mass to parathyroid gland at *b*). Thyroid parenchyma hyperplastic. Hematoxylin and eosin; reduced slightly from mag. × 50.

Fig. 5.—Persisting ultimobranchial tissue cyst lined by stratified squamous epithelium (at *a*). Below (at *c*), note reorganizing metaplastic lesion, which is composed largely of suprabasal cells undergoing repair following acquisition of carotene for six days (Group III). One clear basal cell is indicated at *b*. Note mitotic figure at *d*. Thyroid parenchyma is hyperplastic. Hematoxylin and eosin; reduced slightly from mag. × 200.

Fig. 6.—Persisting cyst (at *a*) similar to that of Figure 5*a*, showing cluster of enlarged, clear basal cells (at *b*) in the process of leaving germinative layers of the epithelium during repair of lesion. Note small, densely staining suprabasal cells on right, indicated by arrows at *c*. Few basal cells can be seen, as they have already contributed to the neighboring thyroid parenchyma. Hematoxylin and eosin; reduced slightly from mag. × 160.




(See legends on opposite page)

Furthermore, this hyperplasia typically predominated in the central portion of the thyroid lobe, and not in the periphery. Thirty-six of the 40 young rats subjected to this deficient diet alone exhibited thyroid metaplasia (90%, Table). This observation was not recorded by Coplan and Sampson [10] in their extensive study dealing with the effect of iodine and vitamin A deficiency on the structure of the thyroid follicle. Without the use of serial sections, however, these lesions may be completely overlooked.

In the current study, rats fed a deficient diet for more than 10 days, without exception, developed multiple cysts lined by stratified squamous epithelium in both lobes of the thyroid gland. These cysts arise directly from presumably normal follicles. The epithelium of the cyst changes by the addition of a superficial layer of squamous cells as it enlarges individually or by fusion with adjacent vesicles of similar character (Figs. 2 and 3). However, extensive cyst formation, which can usually be correlated with progressive thyroid atrophy following prolonged deficiency, may also be due to local hyperplasia in the cyst epithelium (Fig. 7). These cysts are consistently found in the position in which the fetal ultimobranchial body would be expected to lie. This location is typically the center of each lateral lobe of the thyroid gland and/or near the parathyroid gland (Figs. 1 and 4). Moreover, such areas of metaplasia occur many days before symptoms of xerophthalmia, and before any marked degeneration of the thyroid parenchyma.

In concert with the initial hyperplasia of the thyroid during the first half of the experimental period (20 days), germinative, or basal, cells in the epithelial cysts produce large, clear gland-like cells arranged in clumps or small vesicles resembling young thyroid follicles. These new cell groups take a position just outside the cyst wall, where they mingle with the surrounding thyroid parenchyma. During the earlier periods of vitamin A deficiency, such vesicles also may undergo a transformation (through metaplasia) to form accessory cysts lined by stratified squamous epithelium. This was not the case, however, in experimental thyroid glands showing atrophy. The potentialities of such a "thyroid growth center" during

EXPLANATION OF FIGURES 7, 8, 9, 10, 11, 12

Fig. 7.—Large, thin-walled ultimobranchial tissue cyst (upper arrow) occurring in the center of the thyroid lobe of a young rat kept on vitamin A-deficient diet for 40 days. Note concentric mass of desquamated epithelial scales (lower arrow). This cyst is composed largely of suprabasal cells. Thyroid parenchyma atrophic. Hematoxylin and eosin; reduced slightly from mag. × 100.

Fig. 8.—Early stage in transformation of repair in epithelial cyst after feeding carotene for six days (Group II). Original desquamated scales are shown at *a*. Note additional desquamation of superficial, or suprabasal, cells at *b* following acquisition of vitamin A. The epithelial wall of the cyst, which consists largely of basal cell layers, is shown at *c*. Hematoxylin and eosin; reduced slightly from mag. × 200.

Fig. 9.—Later stage in transformation of repair in epithelial lesion following acquisition of carotene for eight days (Group III). Original epithelial desquamation is shown at *a*. Note small vesicle, composed of clear cells (at *b*), which was derived from basal cells in this stratified squamous epithelium. Here the cyst wall is composed mostly of suprabasal cells, a small cluster of which is illustrated at *c*. Hematoxylin and eosin; reduced slightly from mag. × 200.

Fig. 10.—Degenerating mass of suprabasal cells persisting after feeding of carotene for 10 days (Group IV). Note the small, clear-staining follicle (at *a*), which is presumably a recent product of germinal (basal) cells in the epithelium of a reorganizing cyst. Two abortive suprabasal cell vesicles are illustrated at *b*, associated with a larger mass of apparently degenerating cells (*c*). Hematoxylin and eosin; reduced slightly from mag. × 175.

Fig. 11.—Clumps of persisting, partially degenerated suprabasal cells (arrows) following addition of carotene to diet for 10 days (Group III). Basal cells are no longer conspicuous, having contributed to surrounding thyroid parenchyma. Original products of cyst desquamation are shown at *a*. Note mitotic figure, probably within a basal cell, located at *b*. Hematoxylin and eosin; reduced slightly from mag. × 175.

Fig. 12.—Densely staining suprabasal cell mass, persisting one month after acquisition of vitamin A. With the exception of such foci of degenerating cells, no evidence of a previous metaplasia was apparent. Basal, or germinative, cells of the original cyst epithelium were no longer recognizable. Note that suprabasal cells here have produced at least five vesicular formations (arrows), resembling thyroid tissue. These may persist indefinitely; they apparently divide by a process of amitosis. Hematoxylin and eosin; reduced slightly from mag. × 250.

phases of thyroid activation has already been briefly mentioned.[6] This is a concept which will be dealt with more fully in a separate communication.

When vitamin A-deficient rats receive a stock diet containing carotene, the ultimobranchial cysts usually demonstrate an early series of changes leading to their disappearance. These changes were similar to the classic description of Wolbach and Howe [11] in their studies of comparable epithelial manifestations. However, the time required for complete transformation (repair) depends essentially on the severity of the deficiency and the extent of the metaplastic lesion (Table). In Group I, only two animals showed epithelial repair following acquisition of vitamin A. Two other rats were devoid of metaplastic lesions. This suggests a possible total repair in these particular animals following 8 or 10 days on carotene after being subjected to the deficient diet for only 10 days. One animal examined after two days showed no change, although metaplasia was evident. Four rats in Group II demonstrated repair. Since metaplasia was lacking in one animal after 10 days on carotene, it was assumed that total repair had occurred. All rats in Groups III and IV illustrated epithelial repair when fed a stock diet plus carotene for four or more days. The Table indicates the persistence of cysts in the process of repair, even though the animals had been on the corrective diet for 10 days. Lesions that resulted after 30 or 40 days on the deficient diet apparently require the longest period for total repair. Marked hyperplasia of the thyroid parenchyma was observed in all animals that received the restorative diet for more than two days.

During the sequence of repair in these metaplastic cysts, cells which were in the process of keratinization (essentially suprabasal cells) separated (delaminate in masses) from the germinative, or basal, cell layers (Fig. 8, *b* and *c*). The cells at first occupy a position within the center of the cyst. Basal cells, however, leave this unstable epithelium to produce cell clusters that quickly acquire the typical appearance of thyroid tissue. Figures 5 and 6 (*b*) illustrate these germinative cells as an integral part of the stratified squamous epithelium. Figure 9 (*b*) also shows such cells, but in the form of a small vesicle lying outside the epithelial wall of the cyst. In Figure 10 (*a*) one young (recently formed) follicle composed of basal cells can be identified persisting in the absence of any recognizable cystic epithelium. Such basal cells are characterized by a clear, relatively undifferentiated appearance during their incipient stages of transformation into thyroid-like tissue.

The associated suprabasal cells, on the other hand, tend to remain as a core of densely staining cells within the center of the reorganizing mass. It is possible that these unusual cell clumps later disseminate. They are illustrated in Figures 5, 6, 9, 10 (*c*), and 11. Some suprabasal cells undergo complete degeneration and in doing so stimulate a mild leucocytic reaction. Other similar cells, however, are not necessarily differentiated toward keratinization. They divide rapidly (apparently by a process of amitosis) to form aberrant thyroid-like vesicles, which may persist indefinitely. These are the densely staining vesicles demonstrated in Figures 10 (*b*) and 12. The suprabasal cell vesicles shown in Figure 12 occurred in the thyroid of a Wistar rat fed a vitamin A-deficient diet for 35 days. The animal was then given access to vitamin A for one month before autopsy. Partially degenerated suprabasal cell clumps such as these may be responsible, ultimately, for certain kinds of so-called spontaneous thyroid neoplasia. Similar vesicles, remaining after such a long period of repair, were observed in 6 of the 10 experimental rats maintained under the same conditions.

## COMMENT

Steiner [12] has recently called attention to the significance of preexisting metaplastic lesions in the development of certain experimental tumors of the prostate and salivary glands. In the human thyroid gland, various forms of spontaneous adenomata, as well as carcinomata, have been encountered. How-

ever, two examples of mixed squamous cell carcinoma and papillary adenocarcinoma (adenoacanthoma) reported recently are of particular import here (Ross [13]; Halpert and Thuss [14]). Although, according to Jaffe,[4] epithelial metaplasia is the source of the rare squamous cell carcinoma in the human thyroid gland, Klinck and Menk [15] attribute no particular implication to the occasional squamous cell metaplasias in the human thyroid. The origin of malignant tumors of the human thyroid has been, and still is, a matter of considerable speculation.

In 1944, I identified many highly vascular cystadenomata (some papillary) with spontaneous squamous cell metaplasias (thin-walled ultimobranchial cysts composed largely of suprabasal cells) in atrophic thyroids of senile rats.[16] The evidence suggested that these tumors originated from certain densely staining, atypical gland-like cells (presumably suprabasal cells) derived from the epithelium of these cysts. Such cysts are not uncommon in the thyroid gland of rats of various ages, and they can occur in the human thyroid. Although thyroid metaplasia appears to be a precocious manifestation of vitamin A deficiency in young rats, whose liver storage is not great, it also can be produced in adult rats after only a relatively short period of induction (unpublished data). It is possible that foci of spontaneous metaplasia may develop in the thyroids of animals, including man, after only a slight or relative vitamin A deficiency. In the rat, at least, the daily allowance of vitamin A might be increased with benefit.

Since thyroid metaplasia in rats occurs in the position typically occupied by "masked" ultimobranchial tissue, this tissue must be regarded not only as the source of such metaplasia but also as a cell mass somewhat different from the surrounding thyroid parenchyma. Unlike many other organs, the precise site for the development of nests of metaplastic cells is not unpredictable in the thyroid gland. While factors other than vitamin A deficiency also may be instrumental in the production of thyroid metaplasias, ultimobranchial tissue is particularly vulnerable to a lack of this vitamin. McCullough [1] believes that metaplasia commonly occurs in the thyroid gland during vitamin A deficiency, but only in association with epithelial hyperplasia. The concept that ultimobranchial tissue may act in the capacity of a thyroid growth center (Van Dyke [17]) receives some support from McCullough's contention that metaplasia may be prematurely precipitated by secondary factors which increase the rate of cell growth. The marked hyperplasia which was demonstrable in our rat thyroids occurred typically during the early half of the experimental period. While this is compatible with a young, growing thyroid gland, the hyperplasia here was restricted especially to the central portion of each lobe, and it also involved cyst epithelium. Since basophiles of the hypophysis are significantly increased during vitamin A deficiency (Sutton and others [18]), it is possible that thyroid hyperplasias under these conditions may also reflect an increased output of the thyrotropic hormone.

Apparently, the hypophysis is the only other endocrine organ that can develop such metaplastic lesions during vitamin A deficiency. In 1942 Madsen and others [19] described cystic pituitaries in young cattle with a deficiency of vitamin A. These cysts were associated with the area of the pars intermedia. Since there was no evidence of repair at autopsy, following a period of vitamin A therapy, it was presumed that these lesions were permanent. Similar manifestations in the thyroid gland of the rat are essentially reversible.

However, it is important to note that certain aberrant cell remnants of this epithelium may persist indefinitely after periods of repair. Such are the superficial or suprabasal cells in the stratified squamous epithelium of the cystic lesions. Some of these cells undergo only partial (incomplete) degeneration during the process of differentiation toward keratinization. After an appropriate stimulus, such cell masses, remaining as the result of metaplasia, may be the source of certain thyroid neoplasms in

man, as well as in the rat. The realization of the tumor-forming potentialities of such aberrant cell masses in the human being with a history of vitamin A deficiency or some debilitating illness is, therefore, important.

In 1950, studies dealing with experimental thyroid metaplasias suggested a tendency for these lesions to produce mixed epithelial neoplasias.[20] Recent investigations have indicated that metaplastic areas resulting from chronic vitamin A deficiency in adult rats can be induced to form tumors of two essential kinds. Details concerning the origin and manner of development of these experimental thyroid neoplasms will be presented separately.

## SUMMARY

Specific metaplastic lesions in the thyroid gland resulting from vitamin A deficiency are described and demonstrated in the young rat. The evidence indicates that this metaplasia occurs in the precise site occupied by the ultimobranchial tissue component of the thyroid gland and that it is predictable. Thyroid-like vesicles derived originally from this "ultimobranchial body" during embryonic development transform into multiple cysts lined by hyperplastic stratified squamous epithelium. While these changes are not irreversible following vitamin A therapy, certain cell remnants of these cysts or cell proliferations derived from them during their existence may remain after periods of repair. In the present instance, attention is called to the suprabasal cells in this epithelium, which are incompletely differentiated toward keratinization. Such partially degenerated cells can multiply (apparently by amitosis) and reconstitute aberrant vesicles resembling thyroid tissue. These cell clusters can persist indefinitely and may be the source of some spontaneous thyroid tumors in mammals, including man. The origin and manner of development of experimental thyroid neoplasms derived from such metaplastic lesions (ultimobranchial tissue) in the rat will be presented in detail in a subsequent publication.

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# HISTOCHEMICAL STUDIES OF THE EFFECT OF IRRADIATION OF THE PLACENTA

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HISTOCHEMICAL techniques offer means of better understanding of changes produced by ionizing radiation in tissue cells.[1] After irradiation, certain changes in tissue may present reaction patterns different from those revealed by conventional histologic methods. Among the vital processes first presenting themselves for investigation in pursuance of this concept are those of the enzyme systems. Various of these systems are said to be sensitive to irradiation.*

The dehydrogenase enzymes, and succinic dehydrogenase in particular, have shown correlation with sites of cell growth and function in various contexts. In the human female genital tract evidence of dehydrogenase activity has been found to correlate with sites of cell growth in the cervix [4] and with sites of cell proliferation and presumed hormone production in the ovary.[5] In recent years histochemical methods for the demonstration of succinic dehydrogenase have provided means for determining the sites of activity of this oxidative enzyme within cells and tissues.[6] Using such a method in a study of irradiation effect on the ovary,[7] we found that sites of diminution of succinic dehydrogenase activity in general corresponded to regions of tissue damage discernible in conventionally prepared sections.

In the present study the effect of irradiation on the rabbit placenta has been studied, employing histochemical techniques with especial emphasis on localization of succinic dehydrogenase activity. The placenta is a prime example of rapid proliferation of mammalian cells. Its genesis, evolution, and increase in volume are well delineated. To certain of the cells (e. g., syncytiotrophoblast) have been ascribed specific hormone production.[8] The placenta offers for study a complexity of cell types. It should form an excellent test for the study of irradiation effect on rapidly growing cells through histochemical means.

In the present series of midgestational rabbit placentas harvested five days after administration of a tissue dose of 1,200 r, the principal change was that of foci of necrosis and degeneration of the syncytial cells and of perisyncytial cell groups. These cells showed diminution or loss of evidence of dehydrogenase activity and altered pattern of demonstration of alkaline phosphatase activity, as well as cytomorphologic evidence of deterioration.

## MATERIALS AND METHODS

Twelve New Zealand or California strain rabbits having a gestation period of 30 to 32 days were employed. On the ninth day of gestation each animal was given 1,200 r in air in a single dose to an 8 by 6 cm. port over the left horn of the uterus. The factors of irradiation were as follows: constant potential 200 kv. at 18 ma., with a half-value of 0.5 mm. Cu and 1 mm. Al. The distance was 50 cm., with a dosage rate of 50 r per minute. With these factors at a depth of 2.0 cm. the tissue dose is approximately the same as the air dose. Five days

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* References 2 and 3.

later the animals were killed. Placental tissue from right and left uterine horns with adjacent structures was fixed in Zenker's fluid, neutralized solution formaldehyde U. S. P. (formalin), Rossman's fluid,[9] and ice-cold acetone and incubated in neotetrazolium (*p*, *p'*-diphenylene bis 2-[3, 5 diphenyltetrazolium chloride]).[10]

Frozen sections (15μ) of the formalin-fixed blocks were stained with Sudan IV (hematoxylin counterstain) and Sudan black B (Bismarck brown R counterstain) to demonstrate lipid.†

Sections (5μ) of paraffin blocks from the Rossman fluid-fixed tissues were subjected to the periodic acid routine ‡ for glycogen demonstration. Control sections were saliva-digested [9] to remove glycogen.

Sections (5μ) from the acetone-fixed blocks were processed for alkaline phosphatase demonstration.§

Sections from blocks of tissue fixed in formalin, acetone, and Rossman's fluid were stained with hematoxylin and eosin. Sections from tissue fixed in Zenker's fluid were stained with hematoxylin and phloxine.

Blocks of tissue were processed in neotetrazolium to show specific succinic dehydrogenase, endogenous reductase, combined reaction, and negative control.[10] Frozen sections (15μ) were cut. Endogenous reductase is manifested by the positive tissue reaction induced by the presence of various endogenous substrates in the tissue.‖ For specific succinic dehydrogenase demonstration this endogenous activity is destroyed by freezing the tissue, and succinate in excess is then added as a substrate for the reaction.[10]

Before beginning irradiation studies, identical histochemical procedures were carried out on placental and uterine tissues of 12 rabbits of similar strain and identical stage of gestation. These furnished control material for the present study.

## RESULTS

At laparotomy the right uterine horns and contents revealed no macroscopic evidence of irradiation damage. In the left uterine horns, the embryos had in some instances disappeared and in others remained only as disintegrating tissue fragments. The amniotic fluid in the gestations of the left uterine horn had a faint brown tinge, while that from the right side was clear.

Sections of placental and uterine tissue obtained from the right uterine horn conformed in every morphological and cytochemical detail to those from nonirradiated rabbits. Sections from the left uterine horn showed certain changes to be noted.

In material from the right uterine horn, the histological pattern conformed to published reports of studies on placenta and adjacent structures.¶ The reactions of the histochemical techniques in general were similar to those noted by Wislocki and coworkers.[8] In the tissues incubated in neotetrazolium, sites of dehydrogenase activity were indicated by deposition of black formazan crystals and granules in the cytoplasm or by the presence of red microdroplets in the cells.[10] Similar patterns of distribution were revealed by variations in technique designed to show succinic dehydrogenase and endogenous reductase activity.

Periplacental endometrium sections from the left and right uterine horns were identical in morphological and histochemical detail. There was heavy evidence of dehydrogenase activity in the cytoplasm of the glandular epithelium, with much slighter reaction in the stroma. No glycogen was found in the endometrium. At the tips of the glandular epithelial cells small drops of periodic acid-positive material were found. This material resisted saliva digestion; hence it was not glycogen but was some other periodic acid-positive substance, perhaps a mucopolysaccharide. The nuclei and cytoplasm of the glandular epithelial cells and vascular endothelial cells gave considerable alkaline phosphatase reaction. There was less alkaline phosphatase reaction in the stromal cells. Minimal lipid staining was found in the glandular epithelium, and somewhat heavier cytoplasmic lipid reaction occurred in the superficial stromal cells.

Myometrial samples from left and right uterine horns showed no differences. The myometrium revealed evidence of dehydrogenase activity in its fibers. There was moderate nuclear and light cytoplasmic reaction for alkaline phosphatase and none for lipid.

Decidua from the right uterine horn (Fig. 1*A*) manifested cytoplasmic evidence of de-

† Lillie,[11] p. 159.

‡ Lillie,[11] p. 145.

§ Lillie,[11] p. 106, and Gomori,[12] p. 180.

‖ Gomori,[12] p. 150.

¶ References 13 and 14.

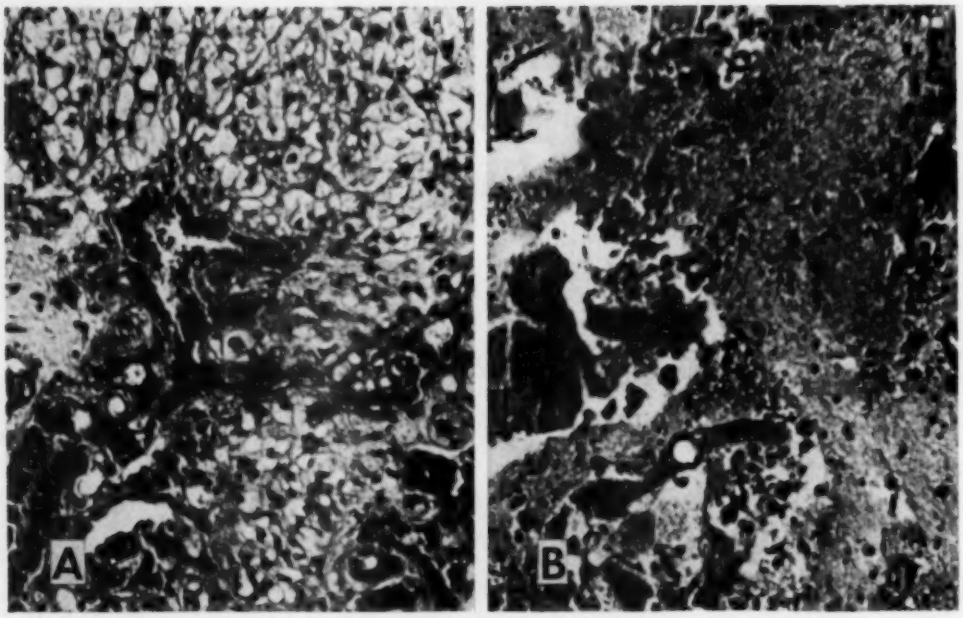
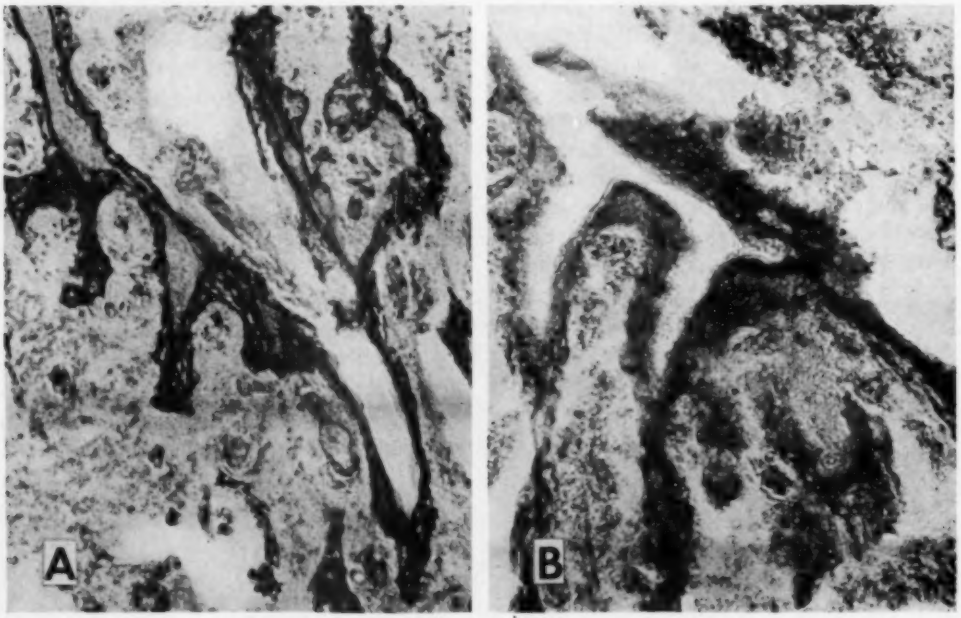



Fig. 1.—*A*, syncytial and decidual cells in rabbit placenta at 14th day of gestation. Tissue is from right (nonirradiated) uterine horn and shows no effect of irradiation. Hematoxylin and eosin, reduced one-fifth from × 270. *B*, comparable area from left (irradiated) uterine horn of same rabbit. Necrosis and disintegration of many of the perisyncytial decidual cells. The syncytial cells show nuclear pyknosis and some cytoplasmic disintegration. Hematoxylin and eosin; reduced one-third from × 270.

Fig. 2 (neotetrazolium section).—*A*, tissue incubated in neotetrazolium with succinate to show sites of succinic dehydrogenase activity. These are seen as cytoplasmic aggregates of formazan crystals or granules. Formazan deposition is very heavy in cytoplasm of syncytial cells, more in peripheral portion than in central cytoplasmic mass. Adjacent decidual cells have moderate formazan deposition. Tissue is from right (nonirradiated) uterine horn. Reduced one-fifth from × 270. *B*, tissue from left (irradiated) uterine horn of same rabbit. Damaged syncytial cells have a much less degree of formazan deposition, indicating dehydrogenase activity. Formazan deposition in adjacent decidual cells is spotty. Reduced one-fifth from × 270.



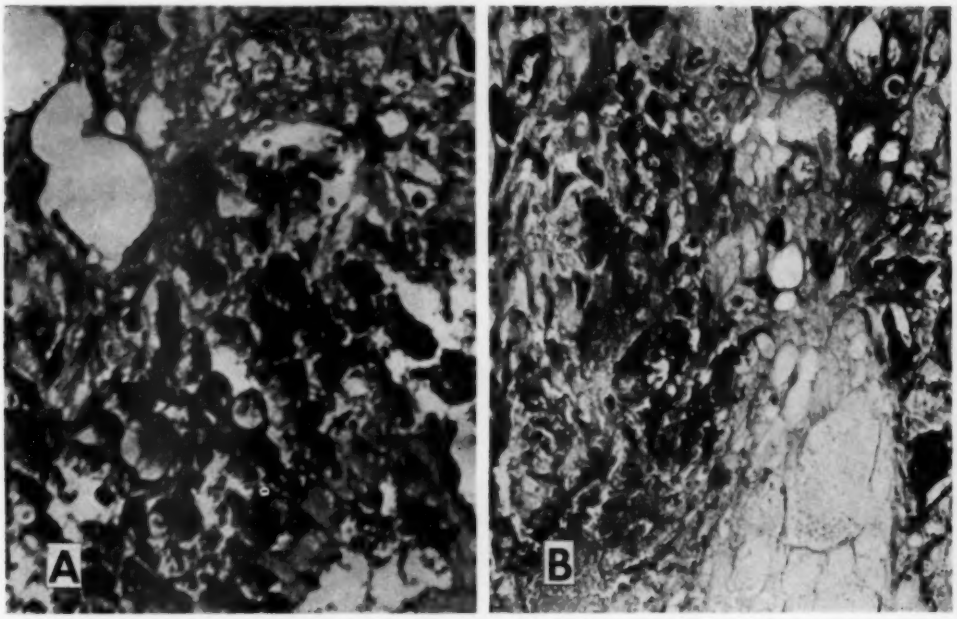
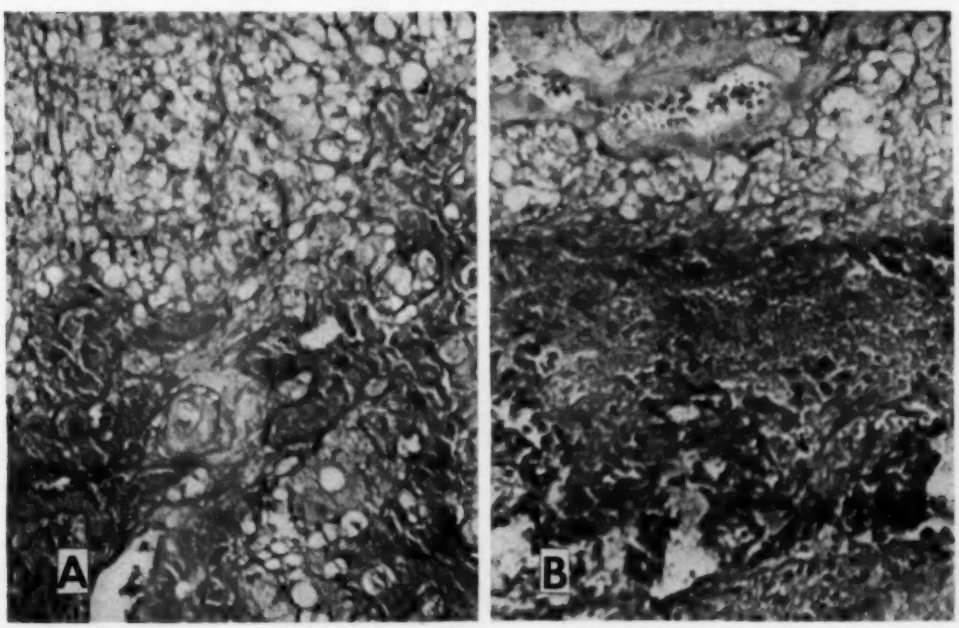



Fig. 3.—*A*, heavy glycogen reaction in cytoplasm of decidual cells. Tissue from right (nonirradiated) uterine horn. Periodic acid stain, nondigested; reduced one-fifth from × 270. *B*, tissue from left uterine horn of same rabbit. Glycogen reaction in damaged decidual cells is absent, irregular in distribution, or weaker. Periodic acid stain, nondigested; reduced one-fifth from × 270.

Fig. 4.—*A*, tissue from right (nonirradiated) uterine horn. Only light basement membrane staining. Periodic acid stain, saliva digested; reduced one-fifth from × 270. *B*, tissue from left (irradiated) uterine horn of same rabbit. Granular material in regions of cell necrosis shows moderate reaction. Periodic acid stain, saliva digested; reduced one-fifth from × 270.



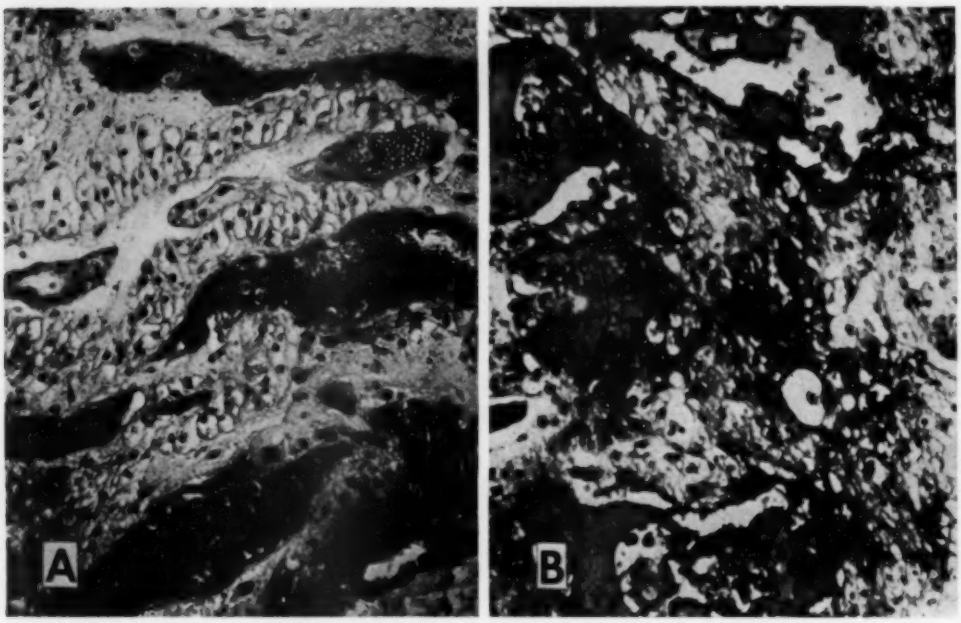
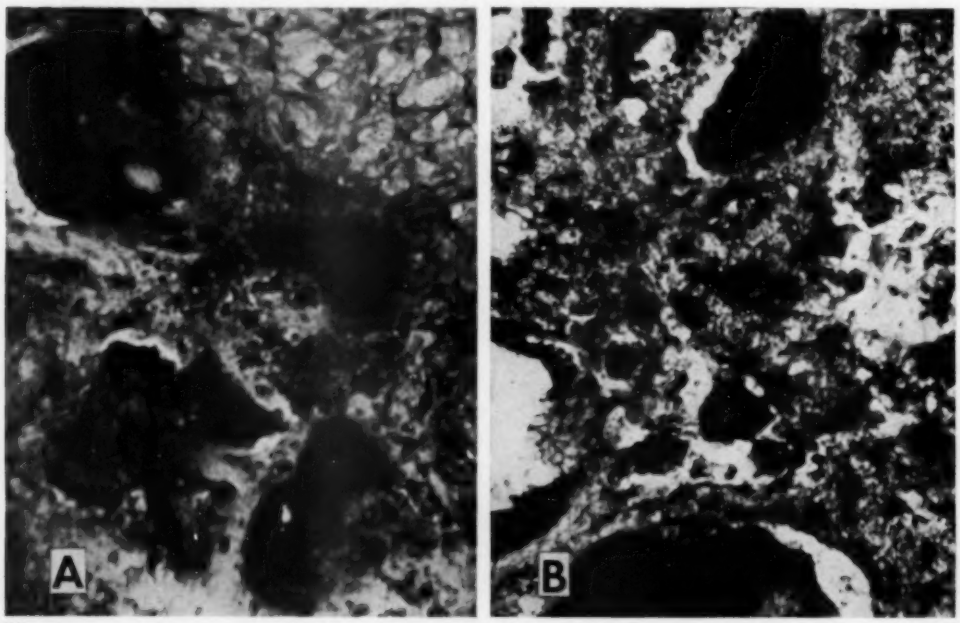



Fig. 5.—*A*, tissue from right (nonirradiated) uterine horn. Moderate cytoplasmic and heavy nuclear reaction in syncytial cells. Heavy nuclear and no cytoplasmic reaction in decidual cells. Alkaline phosphatase stain; reduced one-fifth from × 270. *B*, tissue from left (irradiated) uterine horn of same rabbit. Granular material in regions of cell disintegration has moderate reaction. Alkaline phosphatase stain; reduced one-fifth from × 270.

Fig. 6.—*A*, tissue from right (nonirradiated) uterine horn. Syncytial cells show considerable cytoplasmic reaction. Adjacent syncytial cells have a much weaker staining reaction. Sudan black B stain; reduced one-fifth from × 270. *B*, tissue from left (irradiated) uterine horn of same rabbit. Damaged cells and cellular debris show a considerable reaction with lipid stain. Sudan black B stain; reduced one-fifth from × 270.



hydrogenase activity (Fig. 2*A*). Formazan deposition was somewhat heavier in the lining cells of the uterine sinuses. Glycogen reaction (Fig. 3*A*) was heavy in all decidual cells and absent in the lining cells of the sinuses. Heavy nuclear and moderate cytoplasmic reaction for alkaline phosphatase (Fig. 5*A*) was found in the decidual cells. More evidence of alkaline phosphatase activity was found in the lining cells of the uterine sinuses. A few decidual cells near the syncytial junction contained very light lipid reaction (Fig. 6*A*).

Decidua from the left uterine horn in general manifested similar histological and histochemical findings. However, in the decidual cells adjacent to the syncytial layer, foci of necrosis and cell degeneration were found (Fig. 1*B*). These were from 3 to 6 cell layers thick. In the hematoxylin-and-eosin-stained sections these regions showed nuclear pyknosis or nuclear and cytoplasmic disintegration. The regions of necrosis manifested no evidence of dehydrogenase activity (Fig. 2*B*). They showed much less glycogen staining than did comparable areas in tissue from the right uterine horn (Fig. 3*B*) but had a somewhat more marked granular staining in saliva-digested periodic acid-Schiff sections (Fig. 4*B*). The damaged cells had a moderate cytoplasmic alkaline phosphatase reaction, which was also seen in cellular debris (Fig. 5*B*). The regions of cell degeneration and necrosis gave also a moderate lipid reaction (Fig. 6*B*), considerably stronger than that seen in comparable areas in sections from the right uterine horn.

The syncytial cells in sections from the right side (Fig. 1*A*) had heavy cytoplasmic evidence of dehydrogenase activity (Fig. 2*A*). They gave no evidence of glycogen content but manifested slight evidence of other periodic acid-positive substances. Cytoplasmic and nuclear alkaline phosphatase staining was heavy (Fig. 5*A*). The cytoplasm reacted moderately with lipid stains (Fig. 6*A*).

Approximately one-fourth of the syncytial cells in sections from the left uterine horn showed nuclear pyknosis and some suggestion of cytoplasmic disintegration (Fig. 1*B*). These cells manifested much less evidence of dehydrogenase activity (Fig. 2*B*). In other respects their histochemical reaction pattern showed little change.

The cells of the trophoblast in tissues from the right and left sides showed moderate evidence of dehydrogenase activity, less than the syncytial cells. They contained no glycogen and little other periodic acid-positive substance. They had moderate cytoplasmic and nuclear evidence of alkaline phosphatase activity and reacted faintly to lipid stains.

## COMMENT

Two points must be conceded at the beginning of the discussion. First, the general degree of radiation damage was less than had been anticipated. Both the dose and the time interval were considerably greater than those recently noted by Hicks [16] as sufficient to produce marked gross and microscopic changes in development of the mammalian embryonic central nervous system, including radiation necrosis of vulnerable cells. Up to the 13th day the total volume increase in size of the rabbit placenta, both embryonic and maternal elements, appears considerably greater than that of the embryo proper. Therefore, growth rate as manifested by volumetric increase in size cannot be the most important index of radiation sensitivity. In our material macroscopic evidence of degeneration or disintegration of embryos was apparent; yet microscopic evidence of damage to the placenta was of modest proportion and limited to a narrow range of cellular pattern.

Second, histochemical evidence of radiation damage revealed no greater area of reaction in this study than did standard hematoxylin and eosin sections. The visible evidence of damage was limited to perisyncytial decidual cells and to a proportion of the syncytial cells. Only those cells which showed evidence of nuclear change or cytoplasmic disintegration in hematoxylin and eosin sections revealed variation from the histochemical pattern seen in the control material.

It is of interest that maximum evidence of radiation damage was seen at the junction of fetal and maternal tissue.

It was to be expected that the degenerating and necrotic cells would show diminution of evidence of dehydrogenase activity. Reactability with tetrazolium salts has been used as an index of the viability of plant seeds.[16] Plant seeds irradiated with x-rays and then treated with tetrazolium salts show that the irradiation effect can be detected by changes in the reducing power of the enzyme systems and can be measured by the amount of colored formazan produced.[17] Cellular components of the ovary exhibiting morphologic evidence of radiation damage also showed diminution or absence of evidence of dehydrogenase activity.[7]

Diminution of glycogen reaction and presence of faint nonglycogenic reaction to periodic acid technique appear to be nonspecific manifestations of cell injury. In a previous study[18] we found prominent periodic acid technique staining of damaged ova not due to glycogen. However, this appeared to be a general reaction of atresia in the ova[19] rather than a specific radiation change. Appearance of stainable fat in the cytoplasm of cells in which normally none can be observed is a fairly common indication of rather severe cellular injury.[20] It is often associated with disturbance in functional efficiency of the involved organ and may result from a variety of causative factors.

Histochemical demonstration of sites of dehydrogenase activity, alkaline phosphatase activity, lipid, glycogen, and other periodic acid-positive substances, as seen in this study, has merely elaborated changes discernible in conventional hematoxylin and eosin sections. With the radiation dose and time interval employed, no evidence was found that these histochemical techniques have revealed more widespread or different patterns of radiation damage than would have been delineated without their use.

## SUMMARY

The left uterine horns of 12 rabbits were subjected to external 200 kvp radiation for a single dose of 1,200 r on the ninth day of gestation. Five days later placental tissue from left and right uterine horns was studied for sites of succinic dehydrogenase and endogenous reductase activity, alkaline phosphatase activity, lipid, glycogen, and other periodic acid-positive substances. Tissue from the right uterine horn showed no evidence of radiation damage. In tissue from the left uterine horn, evidence of radiation damage was concentrated in a proportion of syncytial cells and in decidual cells in perisyncytial loci. These manifested degeneration, nuclear pyknosis, disintegration, and cell necrosis. Cytochemically this cellular damage was reflected by diminution in evidence of dehydrogenase activity, appearance of lipid-staining propensities more marked than in control material, granular alkaline phosphatase staining, and nonglycogen type reaction to the periodic acid technique. The decidual cells also showed diminution in glycogen type staining. These reactions appear to be nonspecific manifestations of cellular damage, not peculiar to radiation effect. The cytochemical evidence of radiation damage corresponded in distribution to the cytomorphological manifestation of this reaction.

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# "PARADOXICAL" ANTINECROTIC EFFECT OF CORTISOL

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IT HAS been demonstrated in a variety of experimental conditions that anti-inflammatory hormones (e. g., corticotropin, cortisone, and cortisol*) facilitate the production of necrosis by local irritants.† Presumably, potentially necrotizing substances can penetrate and destroy adjacent tissues more readily than otherwise if they are introduced into the body at a time when the formation of a protective inflammatory barricade is inhibited by antiphlogistic hormones. The object of this communication is to show that, under certain experimental conditions, an apparently paradoxical pharmacologic action, a protection against necrosis, can be obtained by the same type of hormone.

Systemic stress elicits a triphasic general adaptation syndrome, which evolves in three phases, as follows: (*a*) the alarm reaction; (*b*) the stage of resistance, and (*c*) the stage of exhaustion.[2] Correspondingly, if only a circumscribed region of the body is exposed to nonspecific injury, the resulting local stress produces a local adaptation syndrome. This also goes through three stages: (*a*) the local alarm reaction (cellular degeneration, necrosis, and acute inflammation); (*b*) the local stage of resistance (chronic inflammation and cell proliferation), and (*c*) the local stage of exhaustion (degeneration and necrosis).‡ The finding that antiphlogistic hormones facilitate necrosis has been made exclusively in acute experiments, that is, during the local alarm reaction. However, our theory assumed that only the initial necrosis is due to the lack of an adequate "inflammatory barricade," while the terminal necrosis of the local stage of exhaustion was thought to be the result of the wear and tear on tissues caused by inflammation itself. If this concept is correct, it should be possible to prevent the terminal necrosis by suppressing inflammation with the same antiphlogistic hormones that facilitate the production of the initial necrosis.

It has now been demonstrated that this can actually be accomplished, under suitable experimental conditions, with cortisol. This hormone, which has been found to facilitate tissue necrosis during the acute phase of exposure to a nonspecific local irritant, such as croton oil,§ prevents that necrotizing action of the same agent, which otherwise occurs during the terminal phases of inflammation. It was felt that these observations deserve to be reported here because they bear directly upon the rational planning of a pharmacologic treatment of tissue injury.

## MATERIALS AND TECHNIQUES

*Experimental Animals and Their Maintenance.*—In all these investigations, we used female Sprague-Dawley rats, weighing 120 to 155 gm. at

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Merck & Company, Inc., supplied the cortisol-acetate; Ciba Company, Limited, the testosterone and progesterone, and the Schering Corporation, the acetoxypregnenolone used in the experiments.

* In agreement with the suggestion made by Shoppee,[6] the term cortisol is used here in preference to "hydrocortisone," since the term cortisol eliminates confusion with 4,5-dihydrocortisone and obviates the possible implication that "hydrocortisone" is to be regarded primarily as a mere derivative of cortisone.

† References 3 and 4.

‡ References 4 and 5.

§ References 3 and 4.

the beginning of the experiment. They were fed on Purina Fox Chow and given ordinary tap water to drink. Only in the experiment in which adrenalectomized rats were used did we provide 1% NaCl as drinking fluid.

*Topical Stress.*—The "granuloma pouch" technique, which has been described elsewhere in detail,‖ was used to produce topical stress. In essence, the test is performed by injecting 25 ml. of air under the shaved dorsal skin of the rat, this being immediately followed by the injection of some irritant into the resulting air space. In the present experimental series various dilutions of croton oil U.S.P. (in corn oil) were used to cause tissue injury. In all instances the volume of each injection was 0.5 ml. However, the concentration of the croton oil varied and is therefore indicated in the Tables.

If a single injection of a strong irritant is applied under these conditions, histologic evidence of necrosis, in the connective tissue surrounding the air space, becomes manifest within the first few hours. This initial necrosis may actually spread through the whole thickness of the covering skin and become macroscopically visible, by mere inspection of the living animal. With smaller doses, the necrosis does not penetrate during the acute stage, but in the course of a few days the entire lining of the air space is transformed into a strong granulomatous membrane and the air in the cavity is gradually replaced by a hemorrhagic inflammatory exudate. The subsequent course, again, largely depends upon the intensity of the topical irritation. If this is very mild, the entire granuloma pouch, including its exudate, is eventually absorbed and healing occurs without terminal necrosis; if damage is severer, the inflammation gradually progresses toward terminal necrosis and healing occurs only after the fluid contents are evacuated through the resulting skin defect.

As mentioned above, strong irritants (or milder irritants if given with cortisol) tend to cause immediate necrosis, and this had to be avoided if we were to study the terminal stage of reaction to tissue injury. It was necessary, therefore, to commence with highly diluted solutions of croton oil and give higher concentrations (capable of producing an eventual exhaustion necrosis) only after some degree of local tissue resistance had been established through the formation of a granulomatous lining. The details of this procedure varied in our experiments and are outlined in Tables 1 and 2.

*Antiphlogistic Hormone Treatment.*—As an antiphlogistic hormone, we used cortisol acetate in the form of an aqueous suspension of microcrystals (25 mg. per milliliter).¶ This solution was injected directly into the cavity of the granuloma pouch, as indicated in the Tables. In order to avoid the initial, prenecrotic effect of the hormone, the cortisol was never administered during the "local alarm reaction" stage, but only several days after the croton oil pretreatment had had a chance to stimulate the formation of a protective granulomatous membrane. This procedure succeeded in avoiding an immediate necrosis in all instances, so that the granuloma pouches were suitable test objects on which to study whether the inflammatory process will terminate in healing or in necrosis.

*Adrenalectomy.*—In the last experimental series we wanted to examine the effect of cortisol in rats completely deprived of endogenous corticoid hormones. For this purpose bilateral adrenalectomy was performed through the lumbar route, 48 hours before the preparation of the granuloma pouch.

TABLE 1.—*Antinecrotic Action of Cortisol in Intact Rats*

| Group | Croton Oil, 0.5 Ml. | Cortisol, 25 Mg./Ml. | Incidence of Terminal Necrosis |
|---|---|---|---|
| I | 1st day, 0.5%<br>4th day, 3% | ..<br>.. | 50% |
| II | 1st day, 0.5%<br>4th day, 3% | ..<br>4th day, 10 mg. | 0 |
| III | 1st day, 0.5%<br>4th day, 4% | ..<br>.. | 70% |
| IV | 1st day, 0.5%<br>4th day, 4% | ..<br>4th day, 5 mg. | 0 |

## RESULTS

The first two experiments were concerned with a study of the possible antinecrotic action of cortisol in intact rats, and their results are summarized in Table 1.

Each of these experiments was performed on 20 rats, of which 10 received no hormone treatment and 10 were given cortisol. Thus, in the first experiment, Group I acted as a control for Group II and, in the second, Group III, for Group IV. It will be noted that, although the same low dose of croton oil was used throughout for the first, preparatory injection, subsequent treatment differed slightly in the two experiments. In the first series (Group I) 3% croton oil was given on the fourth day, with the view to producing an inflammation conducive to terminal necrosis (Group I), and 10 mg. of cortisol was injected simultaneously with this irritant in the experimental group

‖ References 3 and 4.

¶ Distributed by Merck & Company, Inc.

(Group II). Among the controls, this treatment led to necrosis and evacuation of the pouch contents in 50% of the animals, while the remainder showed no perforation and eventual healing through absorption of the granuloma pouch. Among the cortisol-treated animals, necrosis of the pouch wall was not observed in any instance; indeed, the inhibition of exudate formation and of granulomatous tissue proliferation within the pouch wall was so complete that we regarded this amount of the hormone as exceeding the requirements.

Antinecrotic action of cortisol in the intact rat. In both these rats a granuloma pouch was produced with croton oil, under otherwise identical conditions, except that the animal on the right was given cortisol. Note that, in the control (Group III), a large and characteristically well-delimited, ellipsoid inflammatory focus has developed, which has begun to undergo terminal necrosis (dark region near apex). A few days after this picture was taken, the pouch perforated through the necrotic area and the contents were evacuated. In the cortisol-treated animal (Group IV), inflammation was almost completely prevented and healing occurred without necrosis.

Consequently, in a second experiment, a somewhat higher concentration (4%) of croton oil was administered on the fourth day, while the amount of cortisol was decreased to 5 mg. It will be noted that, here, 70% of the granuloma pouches eventually perforated in the control group (Group III), while in the cortisol-treated rats (Group IV), necrosis was again completely prevented (Figure).

The time at which necrosis and perforation occurred in Groups I and III was rather variable. The earliest case of necrosis was seen in one animal on the 8th day, after the second croton oil injection, and the last perforation occurred only on the 29th day. The cause of this individual variability is not known, but, from our point of view, the fact which must be especially emphasized is merely that there was no instance of an initial necrosis. The latter occurs always during the first 24 hours after the croton oil administration, and only if high concentrations of croton oil are administered without any preparatory treatment with a greater dilution. It is this second type of necrosis that has been shown to be facilitated by antiphlogistic corticoids. It must also be pointed out that in every case the animals were followed until either necrosis with perforation had occurred or the pouch began to show evident signs of involution (decrease in volume, absorption of exudate).

The last experiment was performed on 20 bilaterally adrenalectomized rats, which were again subdivided into 10 control and 10 cortisol-treated animals. It was thought that after ablation of the adrenals, in the complete absence of any endogenous antiphlogistic corticoids, the difference between treated and untreated rats would become even more evident. Furthermore, in this last series, the intensity of the topical croton oil treatment was increased by the administration of three successive doses, the last of which reached a concentration of 10%. In order to prevent the development of an eventually necrotizing inflammation, 5 mg. of cortisol was injected into the air space simultaneously with each of the last two croton oil injections, as outlined in Table 2.

It will be noted that, under these conditions, every one of the granuloma pouches in the control series (Group I) terminated in necrosis with perforation (between the 10th and the 15th day). On the other hand,

all animals receiving cortisol (Group II) were protected against this terminal type of necrosis, and, indeed, even exudation and granuloma formation were only minimal.

In the case of local treatment of inflamed tissue with comparatively large doses of corticoids, the question may be raised, of course, whether the observed effects are actually specific for the particular compound used. In some instances, they could be due to nonspecific local pharmacologic actions of steroids in general, to a chemical combination of the steroid and the irritant (croton oil), which might inactivate the latter, or even to some of the materials used in the preparation of the vehicle for the microcrystalline

TABLE 2.—*Antinecrotic Action of Cortisol in Adrenalectomized Rats*

| Group | Croton Oil, 0.5 Ml. | Cortisol, 25 Mg./Ml. | Incidence of Terminal Necrosis |
|---|---|---|---|
| I | 1st day, 1%<br>3d day, 3%<br>7th day, 10% | ..<br>..<br>.. | 100% |
| II | 1st day, 1%<br>3d day, 3%<br>7th day, 10% | ..<br>3d day, 5 mg.<br>7th day, 5 mg. | 0 |

steroid suspensions. However, these possibilities were eliminated by additional experiments performed under conditions identical with those prevailing in Group II (of our second experimental series), except that progesterone, testosterone propionate, and 21-acetoxypregnenolone were used. All these steroids were administered at the 5 mg. dose level and in the same suspension fluid as cortisol; yet they exerted no antiphlogistic or antinecrotic action. This is in complete agreement with the earlier observations of Rindani,[1] who found 5 mg. amounts of the following steroids to be without antiphlogistic or antinecrotic effect in the granuloma pouch technique, under similar conditions: desoxycorticosterone acetate, Reichstein's Compound S, 21-acetoxypregnenolone, progesterone, and testosterone propionate. It appears reasonable to assume, therefore, that the actions observed in the present experimental series were due to the specific pharmacologic properties of cortisol.

## SUMMARY AND CONCLUSIONS

Experiments on intact and on adrenalectomized rats were performed, using the croton oil granuloma pouch as a test object for the study of local stress situations.

Preliminary experiments had shown that the "immediate necrosis," which tends to occur in adjacent tissues during the first 24 hours after the application of strong irritants, is greatly facilitated by anti-inflammatory hormones.

On the other hand, the necrosis of exhaustion is the usual outcome of prolonged inflammatory responses produced by strong chronic tissue irritation. This "terminal necrosis" is viewed as a direct result of tissue exhaustion, due to the inflammation itself. It is thought to be caused primarily by pressure due to the increasing accumulation of exudate, accompanied by a progressive cicatricial contraction of the granulomatous wall.

In agreement with this interpretation, the terminal necrosis normally elicited by chronic irritation with strong croton oil solutions could be prevented when inflammation was inhibited by topical treatment with cortisol.

This "paradoxical" antinecrotic effect of the anti-inflammatory corticoid was evident both in intact and in adrenalectomized rats.

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# LEIOMYOSARCOMA OF RIGHT URETER

## Report of a Case

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LEIOMYOSARCOMA involving the ureter is a rare neoplasm. Only two previous cases have been reported in the English literature, one by Rademaker,[1] in 1943, and the other by Rossien and Russell,[2] in 1946. A third case, reported by Zahorsky [3] in the Czech literature in 1952, did not include any photomicrographs; hence its acceptability is correspondingly impaired. The following case, to the best of my knowledge, is the third to be recorded in the English literature.

### REPORT OF CASE

A. L., a 60-year-old white woman, was admitted to the United States Naval Hospital, San Diego, Calif., on March 26, 1951, for removal of an orthopedic pin from the right hip. During her hospital stay she developed pain in the right flank. Two intravenous pyelograms showed no function in the right kidney, and two attempts to catheterize the right ureter revealed an obstruction 20 cm. above the urinary bladder, at the level of the fourth lumbar vertebra. Surgical intervention was delayed because of cardiac complications, the evaluation of which showed an old posterior myocardial infarction. Sedimentation rate was elevated, ranging from 25 to 44 mm. in one hour. After her cardiac condition was stabilized, exploratory operation was performed, on May 15. A small firm tumor mass attached to the right ureter approximately 8 cm. below the renal pelvis was found. Hydroureter and hydronephrosis was present above the tumor. The upper one-third of the ureter and the kidney was resected. The patient made an uneventful postoperative recovery, although she still complained of pain in the right flank. She was discharged from the hospital on June 21.

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The opinions presented are those of the author and do not reflect the views of the Medical Department of the U. S. Navy.

The patient was readmitted on Dec. 13, 1951, because of nausea and vomiting, as well as pain in the epigastrium and right upper quadrant. At this time the liver edge was palpable 4 fingerbreadths below the right costal margin and was somewhat nodular and tender. Cephalin flocculation was 1+ in 24 hours and 2+ in 48 hours; serum bilirubin was 0.16 mg. per 100 cc.; thymol turbidity was 4 units; alkaline phosphatase was 3.9 Bodansky units, and total protein was 6.60 gm. per 100 cc., with 4.0 gm. of albumin and 2.6 gm. of globulin, per 100 cc. The patient was discharged from the hospital on Dec. 15, unimproved.

She was admitted again on Jan. 8, 1952, because of left hemiplegia occurring suddenly, two days before entry. She was emaciated at this time and appeared both acutely and chronically ill. The nodular liver was still palpable 4 fingerbreadths below the right costal margin. During the terminal episode the temperature ranged from 97 to 99 F.; pulse rate, from 80 to 100 per minute, and respirations, from 20 to 24 per minute. Ophthalmoscopic examination revealed no papilledema. White blood cell count was 14,000, with 84 polymorphonuclear leucocytes and 16 lymphocytes; red blood cell count was 4,800,000, and hemoglobin was 14 gm. per 100 cc. Although the patient was conscious initially, she gradually became more lethargic and finally, on the morning of Jan. 12, became comatose, with death occurring on Jan. 13.

### PATHOLOGIC EXAMINATION OF SURGICAL SPECIMEN

*Gross.*—The specimen consisted of the right kidney and 11 cm. of attached ureter. The kidney measured 9 by 5 by 3 cm. and weighed 150 gm. It had previously been opened, revealing a markedly dilated pelvis and calyces. A tumor nodule, measuring 3.5 cm. in maximal size, was attached to the ureter 2 cm. above the distal end. This was lobulated and firm in consistency. The cut surface was white and somewhat trabeculated. The ureteral lumen was markedly compressed at the site of the tumor and greatly dilated proximal to it.

*Microscopic.*—Sections revealed that the lumen of the ureter was lined by intact transitional mucosa. The smooth muscle layer

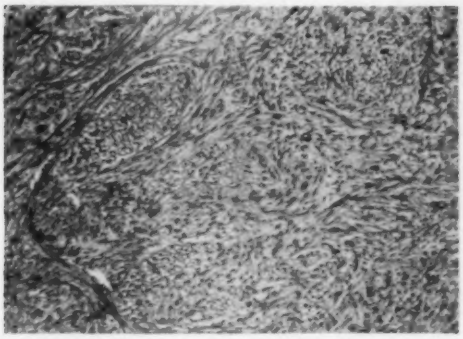
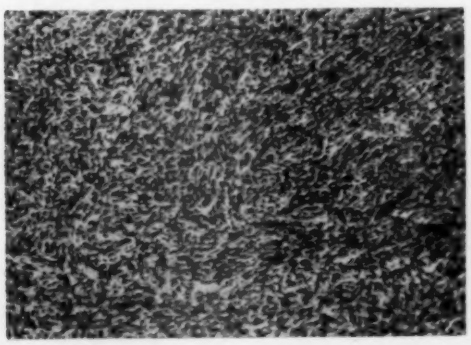


Fig. 1.—Well-differentiated area in the leiomyosarcoma, showing whorling of the smooth muscle fibers in the primary neoplasm; reduced 1/9 from mag. × 100.

was rather well defined. In one area there was a fusion of its smooth muscle fibers with a neoplasm composed of spindle-shaped cells. In some areas these cells were arranged in interlacing whorled bundles. They contained a moderate amount of pink cytoplasm. The narrow elongated nuclei were pale and vesicular and were quite uniform in size and shape (Fig. 1). In other, more cellular, areas the whorled arrangement was poorly defined, and here the nuclei, which were larger and plumper, varied moderately in size and shape and were hyperchromatic, with coarse chromatin granules (Fig. 2). Occasional large irregular black nuclei of bizarre type were scattered throughout. Moderate numbers of mitoses were present (Fig. 3). The cytoplasm of the spindle-shaped

Fig. 2.—More cellular area, showing lack of whorling in the primary neoplasm; reduced 1/9 from mag. × 100.



tumor cells took up a brick-red color with Masson's trichrome stain; hence they were interpreted as myofibrils of smooth muscle type. Variable amounts of dense collagen between the tumor cells took up a blue color.

*Diagnosis.*—Leiomyosarcoma, right ureter, with hydroureter and hydronephrosis.

### Autopsy Report

*Gross.*—Only the pertinent findings will be given. The body was that of an emaciated 61-year-old white woman, measuring 64 in. (162.5 cm.) and weighing an estimated 90 lb. (40.8 kg.). No icterus was noted. A healed surgical incision, 15 cm. in length, was present in the upper right rectus region; an 11 cm. incision, in the lower midline, and a 22 cm., S-shaped one, in the right flank. The liver was firm with large nodules and was palpable 4 fingerbreadths below the right

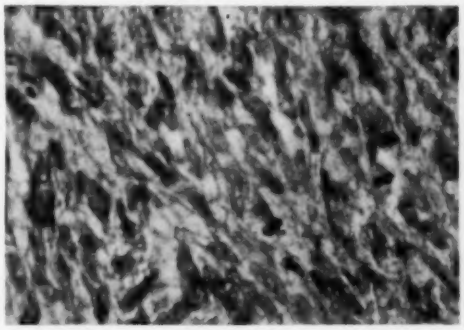


Fig. 3.—High-power magnification, showing mitoses in the primary neoplasm; reduced 1/9 from mag. × 440.

costal margin and 12 cm. below the xyphoid tip. No free fluid was found in the serous cavities. There was surgical absence of the right kidney and proximal ureter down to the pelvic brim.

A firm white tumor nodule, measuring 2 cm. in maximal size, was found in the stump of the right ureter at the pelvic brim. Numerous metastatic tumor nodules were present in both the right and the left lung, which weighed 600 and 425 gm., respectively. The largest tumor nodule was located in the wall of a medium-sized bronchus, just beneath its lumen, in the upper lobe of the right lung

(Fig. 4). These nodules were firm and white, ranging from 0.2 to 2.0 cm. in size. Numerous discrete and confluent tumor nodules, ranging from 0.2 to 8.0 cm. in size, were present in the moderately enlarged liver, which weighed 2,800 gm. (Fig. 4). The large masses bulged beneath the capsule. The smaller firm white nodules were homogeneous throughout. The larger masses, however, presented a bulging white surface at the periphery only. The centers were depressed and dull yellow, with an intervening zone of soft watery gray tissue. A moderate amount of residual hepatic parenchyma remained, which had a mottled, "nutmeg" appearance adjacent to the tumor nodules. A few additional firm white metastatic tumor nodules, averaging 0.6 cm. in size, were present in the head of the pancreas, in the cortex of the left kidney (Fig. 4), and in the bodies of the third and fourth lumbar vertebrae.

The heart was slightly enlarged and weighed 375 gm. Friable, tan vegetations, measuring 1.2 cm. in maximal size, were adherent to the undersurfaces of the aortic cusps, just below the free margin. Most of the firm, dark-red myocardium of the left ventricle averaged 1.6 cm. in thickness. In the apex numerous distinctly demarcated zones of firm gray-white tissue with hyperemic margins replaced the myocardium, the largest of these zones measuring 0.5 cm. in maximal size. Friable, tan-gray mural thrombi were adherent to the apex over the involved area. The distal anterior descending branch of the left coronary artery was plugged by soft, pasty, gray-white material where it curved around the apex. The remainder of the coronary arteries were patent throughout and contained only a few small intramural yellow plaques, without significant narrowing of the lumen.

The spleen, which was small, weighing only 70 gm., contained an irregular soft zone of dull-yellow color with marginal hyperemia, which measured 3 cm. in maximal size. No discrete tumor nodules were present.

The left kidney, which weighed 120 gm., contained, in addition to the tumor nodules, two large irregular depressed lesions, which measured 3 cm. in maximal size. These lesions involved primarily the cortex, which measured as little as 0.2 cm. in thickness. The center of the lesions was dull yellow, while the margins were hyperemic.

The brain, which weighed 1,225 gm., contained a superficial zone of softening in the midlateral portion of the right parieto-occipital region. This zone extended from the Sylvian fissure posteriorly to the level of the posterior horn and involved the entire thickness of the gray matter but only a scanty amount of the underlying white matter. The lesion was soft and friable and dull-yellow to tan. No other similar lesions and

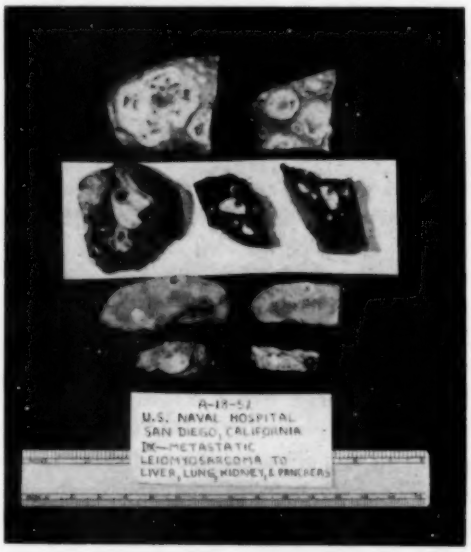



Fig. 4.—Photograph of representative portions of liver, lung, left kidney, and pancreas, showing the metastatic tumor nodules. Note the portion of lung on the left in which a tumor nodule partially surrounds a bronchus, and from which Figure 6 is taken.

no metastatic tumor nodules were present in the remainder of the brain. The vessels of the circle of Willis were thin-walled and patent throughout.

The uterus was small and symmetrical and contained no intramural nodules. No metastatic tumor nodules were found either in the abdominal or in the thoracic lymph nodes.

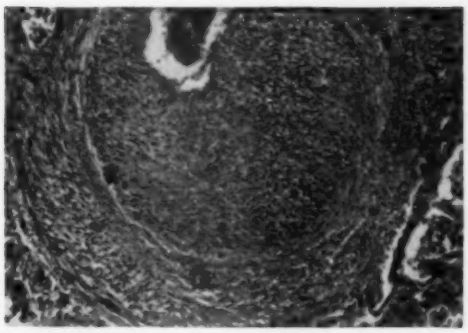
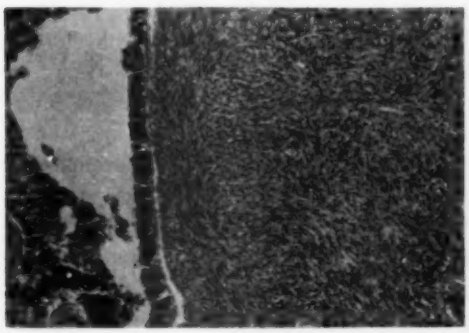


Fig. 5.—Tumor-embolus almost occluding the lumen of a small pulmonary artery; reduced 1/9 from mag. × 100.

*Microscopic.*—Sections of the recurrent ureteral nodule and the metastatic nodules in the lungs (Fig. 6), liver (Fig. 7), pancreas, kidney, and vertebrae all showed a spindle-celled neoplasm with the same histological characteristics as the primary neoplasm. One large tumor nodule was seen in the wall of a bronchus just beneath the respiratory mucosa lining the lumen (Fig. 6). In addition, tumor emboli were found in small pulmonary arteries, with subtotal occlusion of the lumen (Fig. 5). The larger tumor nodules in the liver were necrotic in the center, with replacement by loose, edematous connective tissue. One tumor embolus was found in a small hepatic artery within a portal triad adjacent to a parenchymatous nodule. Masson's trichrome stain was done on representative sections of all these metastases and of the tumor emboli in the pulmonary and hepatic arteries, and, again, a brick-red color was taken up by the cytoplasm of the tumor cells. A scanty amount of blue-staining collagen was present between the tumor cells.

Fig. 6.—Metastasis in the lung infiltrating the wall of a bronchus, just beneath the respiratory mucosa. Note the mitosis in the upper right-hand corner; × 100.



Sections of the aortic valve revealed no change in the cusps. The adherent vegetations were composed of fibrin masses containing some enmeshed leucocytes and red cells. Trichrome stains revealed no viable tumor tissue, and Gram-Weigert stains showed no bacterial clumps in the vegetations.

Numerous sections of heart, spleen, left kidney, and brain revealed many small arteries to be occluded by thromboemboli. Some of these were composed of unorganized fibrin masses containing variable numbers of enmeshed polymorphonuclear cells and red cells. Others were organized by numerous spindle-shaped cells, with intervening dense pink collagen, which took up a blue color with the trichrome stain. No tumor tissue composed of brick-red-staining myofibrils could be found in these thromboemboli. Foci of hyalinized scar tissue and of moderately vascular granulation tissue were seen in the myocardium, as well as foci of acute coagulation necrosis of the muscle fibers, with interstitial edema and leucocytic infiltration. The intact myocardial fibers were somewhat hypertrophied. Unorganized mural thrombi were seen in the left ventricle. Rather dis-

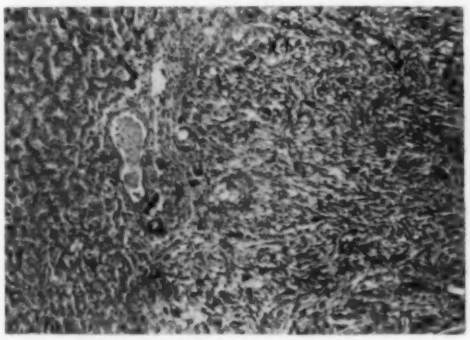


Fig. 7.—Metastasis in the liver adjacent to a portal triad; × 100.

crete areas of coagulation necrosis were seen in the spleen and left kidney, with focal hemorrhages at the hyperemic margins. The kidney, in addition, contained areas of scarring, with hyalinized glomeruli and atrophic tubules. Sections of brain showed cystic encephalomalacia, in which numerous gitter cells were present. Gliosis was a prominent feature at the margin.

COMMENT

All three previously reported cases of leiomyosarcoma involving the ureter occurred in women. Rademaker's [1] case involved the left ureter of a 59-year-old white woman, whose symptoms consisted of a heavy, dull ache in the left upper quadrant for six months and a palpable mass in the left upper quadrant for one month. An intravenous pyelogram showed moderate hydronephrosis on the left side, with the ureter terminating abruptly within a large round sharply outlined soft tissue mass. The neoplasm measured 12.5 cm. in maximal size. At the time of the report the patient was alive and well, without detectable recurrence or metastasis, 14 months after surgery. Rossien and Russell's [2] case involved the right ureter of a 55-year-old white woman, whose symptoms consisted of a "burning sensation" in the right lower quadrant for three months and a 10 lb. (4.5 kg.) weight loss. An "orange-sized" mass was palpable in the right lower quadrant, which was movable and tender. Barium enema showed this mass to be retroperitoneal and medial to the ascending colon. A lobulated neoplasm, measuring 9 by 5 by 4.5 cm., was dissected free from the ureter, but a nephrectomy and partial ureterectomy was not done because the left kidney was found to be a cystic mass whose functional status was open to question. A small metastatic nodule present in the liver was also found. The patient made an uneventful postoperative recovery and remained symptom-free for three months. There was subsequent return of pain in the right lower quadrant, followed by a progressively downhill course. The patient died about two years after surgery because of generalized metastases, but apparently no autopsy was performed. The case reported by Zahorsky [3] involved the right ureter of a 62-year-old white woman.

In 1941 Golden and Stout [4] reported a series of nine retroperitoneal smooth muscle tumors, six of which they considered to be malignant. One of these (Case 24) occurred in a 57-year-old white woman, who had had clinical symptoms for five years and who had a palpable mass in the medial aspect of the right lower quadrant. At operation a firm lobulated mass, measuring 12 by 8 by 6 cm., which was considered to originate in the retroperitoneal tissues in the right lumbar region, was removed. Although the neoplasm was originally thought to be a leiomyoma, because of its well-differentiated histological appearance, it was subsequently interpreted as a malignant leiomyoma because of eventual local recurrence and metastases to the liver. The right ureter was described as encircled by two lobules of the tumor. Although it is possible that the neoplasm in this case had its origin from the smooth musculature of the ureter, it is not being included in this report as a case of leiomyosarcoma of the ureter, since the authors regarded it to be of retroperitoneal origin.

The leiomyosarcoma in the present case involved the right ureter of a 60-year-old white woman and measured 3.5 cm. in maximal size. Death occurred eight months after surgery, which consisted of a right nephrectomy and subtotal ureterectomy. At autopsy recurrent leiomyosarcoma was found in the distal stump of the right ureter at the pelvic brim. In addition, metastases were found in both lungs, as well as the liver, pancreas, left kidney, and third and fourth lumbar vertebrae.

One feature of this case remains unresolved, namely, the etiology of the aortic vegetations. Unfortunately, a culture of fresh material obtained at autopsy was not done. One possibility considered is bacterial endocarditis. It is my personal opinion that this hypothesis is not likely, in view of the clinical picture, the absence of metastatic suppura-

tive abscesses in the heart, spleen, kidney, and brain, and the failure to demonstrate bacterial clumps in Gram-Weigert-stained sections of the aortic valve. This negative evidence certainly does not absolutely exclude this possibility. The second possibility considered is that the lesion was originally a tumor implant which had subsequently become entirely necrotic. The presence of both metastatic tumor nodules and embolic infarcts in the left kidney would be consistent with such a hypothesis. No viable tumor tissue, however, could be demonstrated by means of Masson's trichrome stain either in the aortic vegetations or in the organizing and unorganized emboli in the various involved organs. A third possibility considered, and one that I consider to be the most likely, is that they represent nonbacterial thrombotic vegetations on the basis of severe cachexia.[5] Although the exact etiology of the aortic vegetations remains obscure and undetermined in this case, they played a major role in the cause of the patient's death because of the repeated myocardial infarcts and the terminal cerebral infarct.

SUMMARY

A case of primary leiomyosarcoma involving the right ureter in a 60-year-old white woman is reported, with metastases to the lungs, liver, pancreas, left kidney, and third and fourth lumbar vertebrae.

Death was primarily due to multiple myocardial infarcts and a terminal cerebral infarct secondary to emboli from aortic valvular vegetations, whose exact etiology could not be determined.

Review of the English literature revealed only two well-documented cases, both of which also occurred in elderly white women, 59 and 55 years of age, respectively. The acceptability of a third case reported in the Czech literature is impaired because of the lack of photomicrographs.

Comdr. Charles Hascall (MC), U. S. N., of the Pathology Service, United States Naval Hospital, San Diego, Calif., assisted in taking the photomicrographs; Donald E. Reeves ($HM_3$), U. S. N., of the Pathology Service, prepared the Masson trichrome and Gram-Weigert stains, and William Von Allmen ($HM_3$), U. S. N. R., of the Medical Photography Service, prepared the gross photographs and developed the photomicrographs.

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# IN VITRO DEHYDROGENASE ACTIVITY OF NORMAL AND PATHOLOGICAL LYMPH NODES

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With the Technical Assistance of Jean Watts, B.A.

IT IS WELL known that the microscopic structure of lymph nodes may vary greatly in association with different physiological and pathological states. In an attempt to study such changes, various investigators have utilized histochemical techniques for the visualization of acid and alkaline phosphatases, mucopolysaccharide, deoxyribonucleic and ribonucleic acid, lipid, and glycogen.* These studies indicated that histochemical alterations may be observed in association with diverse pathological states. It therefore seemed desirable to extend such observations with particular reference to the in vitro dehydrogenase activity of lymph nodes in health and disease.

Our previous studies with 2,3,5-triphenyltetrazolium chloride (TTC) as a histochemical and quantitative tool indicated that it should be suitable to an investigation of such metabolic activity in lymphoid tissues.[3] In addition, the effect of fluoride on the dehydrogenase activity was also studied, since we had previously observed that the dehydrogenase activity of tumor tissue tended to be stimulated by fluoride, whereas the homologous control tissues were either inhibited or stimulated to a slighter degree.[4]

## MATERIAL AND METHODS

In this investigation, lymph nodes from 36 cases were studied. The tissues were received from the operating room of the Flower and Fifth Avenue Hospitals within 10 minutes of removal. The in vitro dehydrogenase activity was determined both endogenously ($R_o$) and in the presence of fluoride ($R_f$), according to our previously described techniques.[3] In addition, portions of the tissues which had been incubated in TTC with and without fluoride were taken for frozen section examination to determine the site and type of TTC reduction.

## RESULTS

*Quantitative Studies.*—In Tables 1 and 2, we have listed the individual values obtained for the intensity of TTC reduction by the tissues studied. It will be noted that there was a decided overlap in the individual endogenous values found in the different groups, as well as variability among the values within the groups. Furthermore, the mean values for the different groups of nodes were not appreciably different.

TABLE 1.—*In Vitro Dehydrogenase Activity of Tissue Slices from Non-Neoplastic Lymph Nodes*

| Case No. | $R_o$ | $R_f$ |
|---|---|---|
| Controls | | |
| 895-52 | 2.89 | 4.34 |
| 3763-52 | 7.00 | .... |
| 4064-52 | 4.04 | 3.95 |
| 4316-52 | 7.32 | 10.45 |
| 765-53 | 5.06 | 6.34 |
| 987-52 | 6.38 | 8.39 |
| 4018-52 | 7.37 | 7.88 |
| 206-53 | 8.92 | 11.28 |
| 2891-53 | 5.25 | 7.89 |
| 570-54 | 7.73 | 9.66 |
| Mean | 6.19 | 7.79 |
| Tuberculosis | | |
| 5105-52 | 10.11 | 9.21 |
| Sarcoidosis | | |
| 2056-54 | 8.90 | 11.38 |

Examination of the $R_f$ values revealed that the mean values in the three neoplastic groups were very similar, viz., Hodgkin's disease, 11.80; lymphosarcoma, 11.16; chronic lymphatic leukemia, 11.49, and at least 40% greater than the mean for the control group,

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This study was aided by grants from the Helen Andreadis Foundation and the Leukemia Research Foundation.

* References 1 and 2.

TABLE 2.—*In Vitro Dehydrogenase Activity of Tissue Slices from Neoplastic Lymph Nodes*

| Case No. | $R_o$ | $R_f$ |
|---|---|---|
| Lymphosarcoma | | |
| 4367-51 | 6.25 | 7.90 |
| 2204-52 | 7.97 | 14.90 |
| 2758-52 | 10.25 | 15.80 |
| 4757-52 | 8.35 | 7.25 |
| 1405-53 | 9.32 | 12.40 |
| 3398-53 | 11.74 | 14.00 |
| 527-54 | 6.53 | 8.98 |
| 722-54 | 8.31 | 13.02 |
| 997-54 | 7.64 | 6.16 |
| Mean | 8.48 | 11.16 |
| Hodgkin's disease | | |
| 2842-51 | 8.75 | 10.45 |
| 4358-51 | 9.05 | 9.45 |
| 2756-52 (s) | 7.53 | 9.23 |
| 2756-52 (d) | 10.15 | 11.40 |
| 3468-52 | 7.90 | 14.30 |
| 4836-52 | 7.33 | 8.82 |
| 390-53 | 8.13 | 13.25 |
| 1404-53 | 8.93 | 9.73 |
| 3305-53 | 12.84 | 16.37 |
| 45-54 | 9.99 | 13.50 |
| 2365-54 | 7.40 | 12.42 |
| 2401-54 | 9.53 | 12.02 |
| Mean | 9.00 | 11.80 |
| Chronic lymphatic leukemia | | |
| 4252-51 | 4.50 | 10.20 |
| 2339-54 | 6.60 | 12.78 |
| Mean | 5.55 | 11.49 |

(s) indicates superficial nodes; (d), deep nodes.

7.79. It will also be seen that an $R_f$ value equal to or greater than 10.00 γ per milligram was found in only two of the nine control determinations. On the other hand, this value was exceeded in 5 of the 9 lymphosarcoma cases, in 8 of the 12 Hodgkin's cases, and in both of the chronic lymphatic leukemia cases tested.

In Figure 1, we have indicated the relationship found between the $R_o$ and the $R_f$ values in the individual cases. In the nongranulomatous (control) nodes there appeared to be a linear relationship between these two values. However, in the various neoplastic nodes studied there was a greater degree of variability in this relationship. In the two chronic lymphatic leukemia cases, three of the Hodgkin's cases, and three of the lymphosarcoma cases, the ratio of the $R_f$ to the $R_o$ value was greater than that observed in the control group. However, in the remaining cases this ratio was similar to or less than that observed in the control group. These findings are in keeping with our previous observations of decided metabolic variability in the dehydrogenase activity of tumor tissues.†

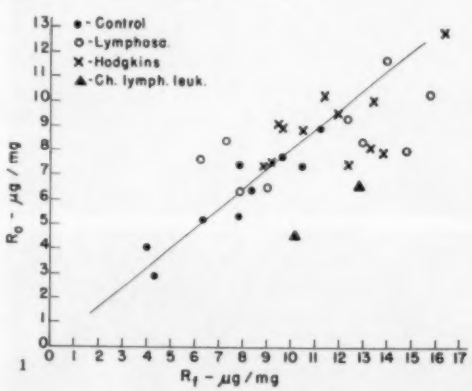
*Histochemical Studies.*—Examination of the frozen sections prepared from the control and pathological nodes revealed a definite tendency for the formazan deposition to be similar in type in the individual cases comprising a group. However, distinct and characteristic histochemical differences were noted among the different groups.

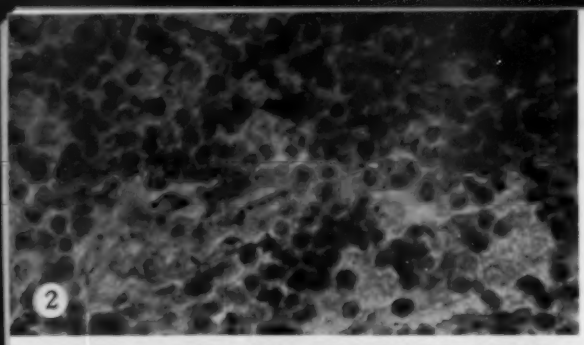
Control Nodes: The sections from the non-neoplastic lymph nodes were characterized by (*a*) elongated formazan crystals which appeared to be within the cytoplasm of the stromal reticulum cells; (*b*) extracellular irregular formazan crystals; (*c*) minimal staining of the lymphocytes of the pulp and follicles; (*d*) scattered macrophages, which showed irregular plate-like crystals in their cytoplasm, and (*e*) the appearance of coarse granular staining in the reticuloendothelial cells and some elongated crystals in the capillary endothelium after exposure to fluoride. In some cases fluoride also seemed to cause a perinuclear formazan deposition in some of the lymphocytes.

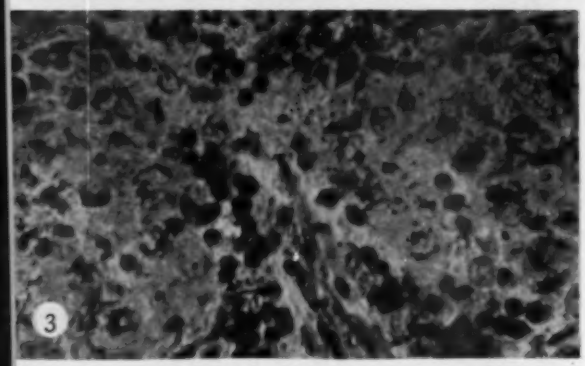
Hodgkin's Disease: The formazan deposition in the Hodgkin's lymph node sections

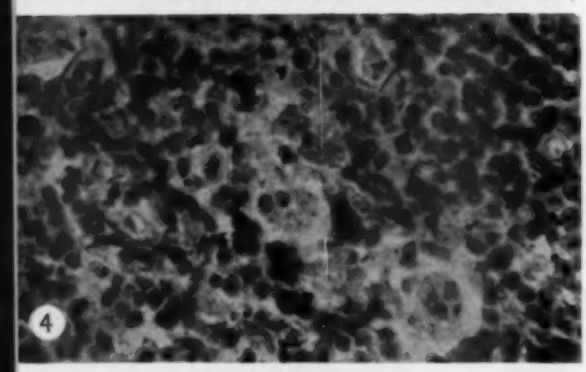
† References 5 and 6.

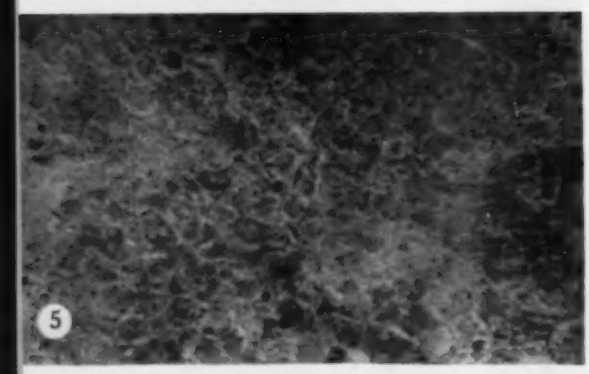
Fig. 1.—$R_o$ and $R_f$ values in control and neoplastic lymph nodes. Note deviation from linear relationship in neoplastic group.

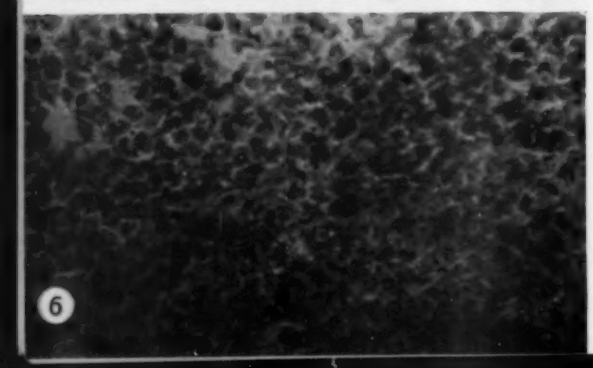












was rather similar to that observed in the control slices. However, there did appear to be a greater intensity of staining of the stromal elements. The reticulum cells showed intracytoplasmic coarse rod-shaped crystals after exposure to fluoride, while the Reed-Sternberg cells and large reticulum cells showed a few coarse elongated crystals. Some coarse granulations were also noted in some of the lymphocytes.

Lymphosarcoma: The lymph node sections from the lymphosarcoma cases were characterized by minimal staining of the stromal elements and on intracytoplasmic fine granular formazan deposition in the tumor cells. In some cases, the formazan appeared as fine, red-stained, lipid-like granules. Exposures to fluoride intensified the staining but did not change its character.

Chronic Lymphatic Leukemia: Lymph node sections from cases of chronic lymphatic leukemia were characterized by moderately coarse irregular formazan granules in the cytoplasm of the lymphocytes. The staining was accentuated and made coarser after exposure to fluoride. There was no appreciable staining of the stromal elements.

Sarcoidosis: The reduction of TTC by lymph nodes involved by sarcoidosis was of two main types: (*a*) the uninvolved areas of lymph node tissue, in which the staining was similar to that observed in the nonsarcoid (control) nodes, and (*b*) the sarcoid granulomas, which were characterized and readily distinguished from the background lymphoid tissue by a prominent staining. This staining consisted of elongated delicate red formazan crystals, which seemed to surround the epithelioid cells of the granuloma.

Fig. 2.—Control node. Note delicate elongated formazan crystals in stroma and lack of staining of lymphocytes.

Fig. 3.—Sarcoid lesion showing prominent formazan deposition in specific granuloma.

Fig. 4.—Hodgkin's disease. Formazan deposition similar to that in control node, some formazan crystal in reticulum and Reed-Sternberg cells.

Fig. 5.—Lymphosarcoma, reticulum cell type. Note punctate granulation in cytoplasm.

Fig. 6.—Chronic lymphatic leukemia. Numerous coarse granules in cytoplasm of leukemic cells.

In some of the cells, such staining appeared to be concentrated around the nucleus. No appreciable granular staining was observed.

Tuberculosis: As with the sarcoid nodes, the uninvolved areas stained in a fashion similar to that of the controls. The formazan deposition in the noncaseous tubercles was similar to but somewhat coarser than that found in the sarcoid granulomas. In the caseous foci, there was no TTC reduction except at the edges, where the oriented epithelioid cells showed a faint lipid-granular type of staining. This staining was accentuated after exposure to fluoride.

## COMMENT

It is evident from the foregoing observations that the in vitro dehydrogenase activity of lymph nodes varies with their physiological state and pathological involvement. This is suggested by the quantitative data obtained and more clearly demonstrated by the histochemical studies. From the standpoint of the metabolic activity of the cellular components of lymph nodes, it would appear that the lymphocytes of the various control nodes—Hodgkin's disease, sarcoidosis, and tuberculosis—do not actively reduce TTC under the conditions of this study. We have also observed a similar lack of active TTC reduction by the lymphocytes in extranodal inflammatory foci and in the normal spleen. In contrast, however, the lymphocytes of lymph nodes involved by chronic lymphatic leukemia and by lymphosarcoma of both the lymphocytic and the reticulum cell type show an active intracytoplasmic TTC reduction. It should also be mentioned that we have observed active TTC reduction by the lymphocytes in spleen slices from cases of hypersplenism. In addition, the lymphocytoid cells of two cases of thymoma both showed well-defined intracytoplasmic formazan granulations, particularly after exposure to fluoride. These observations clearly demonstrate that lymphocytes may vary markedly in their metabolic activity without any appreciable microscopically demonstrable structural change. It is also of interest to note that from a histochemical standpoint, by the methods employed, the lymphocyte in the Hodgkin's node is similar to that in the control node and different from that in chronic lymphatic leukemia and lymphosarcoma. Whether the metabolic alterations in the lymphosarcoma and leukemia nodes are quantitative or qualitative cannot be clearly determined from the present data. In this connection, it should be noted that Ackerman and co-workers,[2] in a histochemical study of nucleoproteins, carbohydrates, lipids, and phosphatases, found evidence of quantitative rather than qualitative alterations in the lymphocytes of neoplastic nodes as compared with control nodes.

The reticulum cells of the control and pathological nodes also exhibit certain differences in their dehydrogenase activity. In the control node, a good part of the total dehydrogenase activity appears to be due to the stromal reticulum cells. The TTC reduction by these cells was visualized as elongated formazan crystals. In sections from the sarcoid and tuberculous nodes, this type of staining was prominent in association with the epithelioid cells of the granulomas. In Hodgkin's disease, elongated formazan crystals also appear to be associated with the proliferating stromal reticulum cells and clasmatocytic types of reticulum cells. In addition, however, there is a tendency for coarse irregular granules to appear after exposure to fluoride. A similar but perhaps less marked tendency was also noted in the Reed-Sternberg cell. In contrast, the reticulum cell lymphosarcoma nodes showed minimal TTC reduction by the stromal reticulum cells but did show a discrete granular type of intracellular formazan deposition. This marked difference between the reticulum and Reed-Sternberg cells in Hodgkin's disease and the tumor cells in reticulum cell lymphosarcoma is in contrast to the observations of Ackerman and co-workers.[2] These authors, utilizing the histochemical techniques mentioned above, found very little difference between the modified reticulum cells of Hodgkin's disease and the cells of the reticulum cell sarcoma.

It is of interest to note that in the present histochemical studies both the lymphocytes

and the reticulum cells of the Hodgkin's nodes behaved very similarly to those of the control nodes and differently than the tumor cells of lymphosarcoma and chronic lymphatic leukemia. However, we are not prepared to state whether these data may be properly used to support the contention that Hodgkin's disease is a non-neoplastic granulomatous disease.[7] Certainly, additional studies appear indicated.

The data obtained in the present study suggest that while certain aspects of the metabolic activity of lymph nodes may serve to characterize them in relation to the histopathological diagnosis, the existence of variability within the groups should not be overlooked. Such variability was particularly evident in regard to the quantitative studies on the fluoride effect on the dehydrogenase activity. It is hoped that further studies will clarify the significance of these findings in terms of the nature and behavior of these diseases.

SUMMARY

The in vitro dehydrogenase activity of control and pathological lymph nodes was visualized histochemically and measured quantitatively by the use of 2,3,5-triphenyltetrazolium chloride (TTC). The quantitative studies did not reveal any significant difference between the endogenous activity of control lymph nodes and the involved nodes of lymphosarcoma, Hodgkin's disease, and chronic lymphatic leukemia. In the presence of fluoride, there was a definite tendency for the tumor nodes to show a greater reduction of TTC per milligram of tissue.

The histochemical studies revealed distinct differences in the type and cellular localization of reduced TTC between the nodes of the control cases and the leukemic and lymphosarcomatous nodes. The Hodgkin's nodes appear more similar to the controls. Observations on sarcoid and tuberculous nodes revealed an active TTC reduction by the specific granulomas.

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## Scientific Exhibits

# FUNGOUS DISEASES

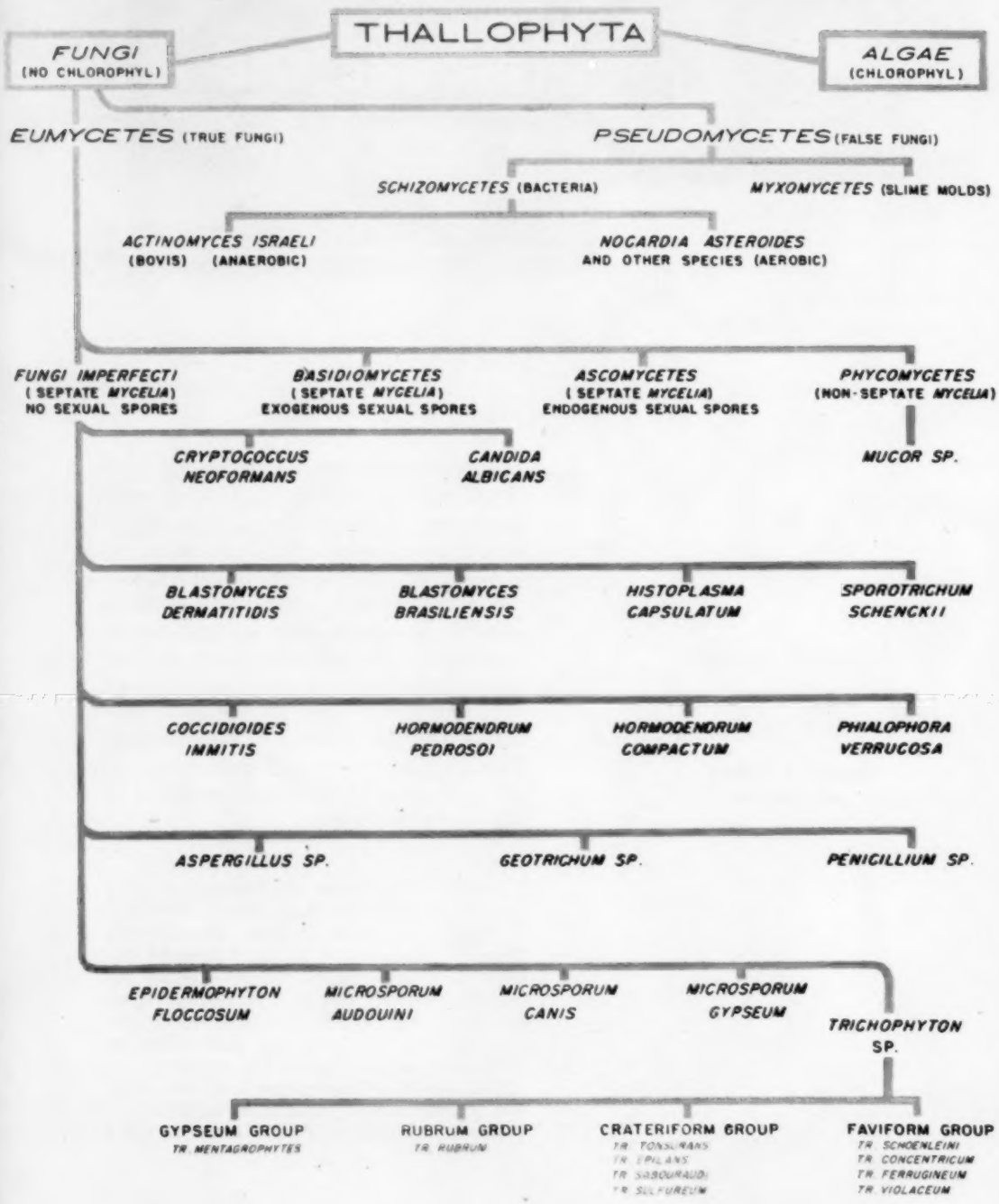
EMMA S. MOSS
ALBERT L. McQUOWN
AND
ROBERT S. COOKE
NEW ORLEANS

FUNGOUS diseases occur world wide. A knowledge of the mycoses, of their signs and symptoms, and of correct treatment is vital for the modern clinician. He is the person who considers them in the differential diagnosis. The definitive diagnosis can only be accomplished in the pathological laboratory. It is imperative that an accurate diagnosis be made, as the treatment varies with each etiologic agent and specific therapy is now available for many of these diseases. This exhibit presents and summarizes the principal points of the diseases produced by the pathogenic fungi.

Director, Department of Pathology, Charity Hospital of Louisiana at New Orleans, and Clinical Professor of Pathology, Louisiana State University School of Medicine (Dr. Moss); Pathologist, Our Lady of the Lake Hospital, Baton Rouge, La., and Clinical Assistant Professor of Pathology, Louisiana State University School of Medicine (Dr. McQuown); Assistant Pathologist, Charity Hospital of Louisiana at New Orleans (Dr. Cooke).

(Reproduced with permission from Moss, E. S., and McQuown, A. L.: Atlas of Medical Mycology, Baltimore, Williams & Wilkins Company, 1953.)

# CLASSIFICATION



# METHODS OF DIAGNOSIS

## CLINICAL SIGNS AND SYMPTOMS (MOST IMPORTANT OF ALL)

AGE INCIDENCE
SEX INCIDENCE
LOCATION OF LESIONS
DURATION OF LESIONS
APPEARANCE OF LESIONS
OCCUPATIONAL HISTORY
GEOGRAPHIC LOCATION (IMPORTANT IN SOME INFECTIONS)

## LABORATORY DIAGNOSIS

DIRECT EXAMINATION (PUS, EXUDATES AND SCRAPINGS) STAINED AND UNSTAINED WET MOUNTS, SIMPLEST-MOST IMPORTANT.
CULTURAL METHODS. NECESSARY FOR DEFINITIVE DIAGNOSIS.
HISTOLOGICAL EXAMINATION. GRANULOMATOUS CHANGES, SPORES OR COLONIES OF FUNGI MAY BE PRESENT.
CUTANEOUS TESTS. USEFUL IN COCCIDIOIDOMYCOSIS, BLASTOMYCOSIS, SPOROTRICHOSIS, HISTOPLASMOSIS AND TRICHOPHYTOSIS.
ANIMAL INOCULATION. USEFUL IN SOME SYSTEMIC INFECTIONS.
FERMENTATION TESTS. RESULTS USUALLY POOR.
AGGLUTINATION TESTS. USEFUL IN MONILIASIS AND BLASTOMYCOSIS.
PRECIPITIN AND COMPLEMENT FIXATION TESTS. USED WITH SYSTEMIC MYCOSIS--VALUELESS FOR SUPERFICIAL FUNGI.
FUSION OF MYCELIA (RESEARCH). ONLY STRAINS OF SAME SPECIES WILL FUSE WHEN ALLOWED TO MINGLE.

## OTHER AIDS

ROENTGEN EXAMINATION. USEFUL FOR PULMONARY AND BONE LESIONS.
FILTERED ULTRAVIOLET RADIATION. FOR CUTANEOUS FUNGI.
THERAPEUTIC TEST. USED PRIOR TO IDENTIFICATION OF ETIOLOGIC AGENT. SHOULD NOT BE USED.

# PROCEDURES FOR MYCOLOGICAL DIAGNOSIS

## SPECIMEN

RESULTS MORE SATISFACTORY WHEN SPECIMEN OBTAINED PRIOR TO TREATMENT.
CLEAN SKIN WITH ACETONE FOR SKIN SCRAPINGS.
AVOID CONTAMINATION.

## DIRECT MICROSCOPIC EXAMINATION OF SPECIMEN

WET PREPARATION MOST USEFUL.
- UNSTAINED - EXAMINE WITH REDUCED LIGHT.
  - USE MATERIAL AS OBTAINED.
  - SUSPEND IN SALINE IF NECESSARY.
  - CLEAR IN KOH IF NECESSARY.
- STAINED - ADD AQUEOUS STAIN FOR CONTRAST.
- INDIA INK - TO DEMONSTRATE CAPSULES AND DIFFERENTIATE YEAST-LIKE ORGANISMS.

FIXED STAINED PREPARATIONS.
- SCHIFF'S PERIODIC ACID, (HOTCHKISS - McMANUS MODIFICATION).
- HEMATOXYLIN AND EOSIN.
- GRAM'S
- ACID FAST

## METHODS FOR CULTURES

MEDIA - ROUTINE:
- LITTMAN'S OXGALL: SKIN SCRAPINGS, HAIRS, SPUTUM, FECES, URINE, CONTAMINATED EXUDATES FROM SINUSES, ULCERS AND ABSCESSES.
- SABOURAUD'S GLUCOSE AGAR: UNCONTAMINATED TRANSUDATES AND EXUDATES; ALSO FOR ANY MATERIAL SUSPECTED OF CONTAINING *ACTINOMYCETES* AND *NOCARDIA SP*.

MEDIA - SPECIAL:
- BLOOD AGAR: *HISTOPLASMA* AND YEAST PHASE OF SYSTEMIC FUNGI.
- BRAIN VEAL INFUSION: AGAR SHAKE TUBES FOR *ACTINOMYCES ISRAELI*.
- CORN MEAL OR CHLAMYDOSPORE AGAR: NECESSARY FOR DIFFERENTIATION OF *CANDIDA ALBICANS*.

## TEMPERATURE OF INCUBATION

20° C.
- CULTURES ON LITTMAN'S OXGALL MEDIUM, SABOURAUD'S GLUCOSE AGAR, CORNMEAL AGAR, AND CHLAMYDOSPORE AGAR.

37° C.
- BLOOD AGAR.
- BRAIN VEAL INFUSION AGAR.
- ANY ORGANISM THAT CAN BE CONVERTED FROM MOLD TO YEAST ON SUBCULTURE.

## TIME ROUTINE

OBSERVE 3 TO 21 DAYS.

## IDENTIFICATION

CHARACTERISTIC COLONY GROWTH ON MEDIUM OF CHOICE.
WET PREPARATION. CHARACTERISTIC SPORES AND MYCELIUM.
SLIDE CULTURE. CHACTERISTIC GROWTH WITH DEVELOPMENT OF TYPICAL SPORES AND HYPHAE.

# CONTAMINANTS

USUALLY GROW MORE RAPIDLY THAN PATHOGENS.

SPREAD RAPIDLY ON SABOURAUD'S MEDIUM.

TEND TO OUTGROW PATHOGENS ON SABOURAUD'S MEDIUM.

ON LITTMAN'S OXGALL MEDIUM TEND TO PRODUCE SMALL, WELL ISOLATED, NON-SPREADING COLONIES.

MAY BE TRUE CONTAMINANTS---- AIR-BORNE.

MAY BE SECONDARILY PRESENT IN LESIONS DUE TO PATHOGENIC FUNGI.

OCCASIONALLY MAY BE ETIOLOGIC AGENT.

# MEDIA

LITTMAN OXGALL AGAR: IDEAL FOR PRIMARY ISOLATION ESPECIALLY FOR THE DERMATOPHYTES.

SABOURAUD GLUCOSE AGAR: GOOD MEDIUM FOR ISOLATION FROM UNCONTAMINATED MATERIAL. MAY BE IMPROVED BY ADDITION OF ANTIBIOTICS. EXCELLENT FOR STOCK CULTURES.

CORN MEAL AGAR AND CHLAMYDOSPORE AGAR: USEFUL IN IDENTIFICATION OF *CANDIDA ALBICANS*.

BLOOD AGAR: GOOD FOR CONVERSION TO YEAST-LIKE PHASE AT 37°C. EXCELLENT FOR ISOLATION OF *HISTOPLASMA CAPSULATUM*.

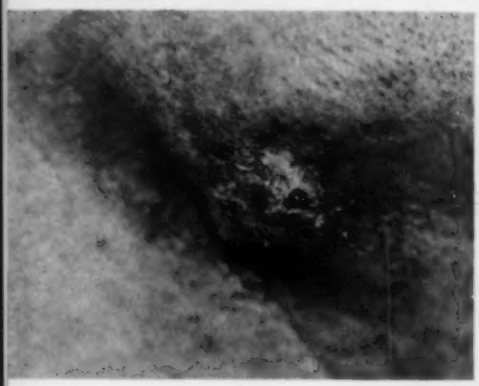
BRAIN HEART INFUSION BROTH WITH AGAR: GOOD FOR ISOLATION AND GROWTH OF *ACTINOMYCES ISRAELI* (ANAEROBIC).

ANY ANTIBIOTIC OR VARIOUS ANTIBIOTICS MAY BE ADDED TO THE ABOVE MEDIA FOR REDUCTION OF CONTAMINANTS. PREPARED DEHYDRATED MEDIA OF THE ABOVE TYPES MAY BE PURCHASED COMMERCIALLY.

# ACTINOMYCOSIS

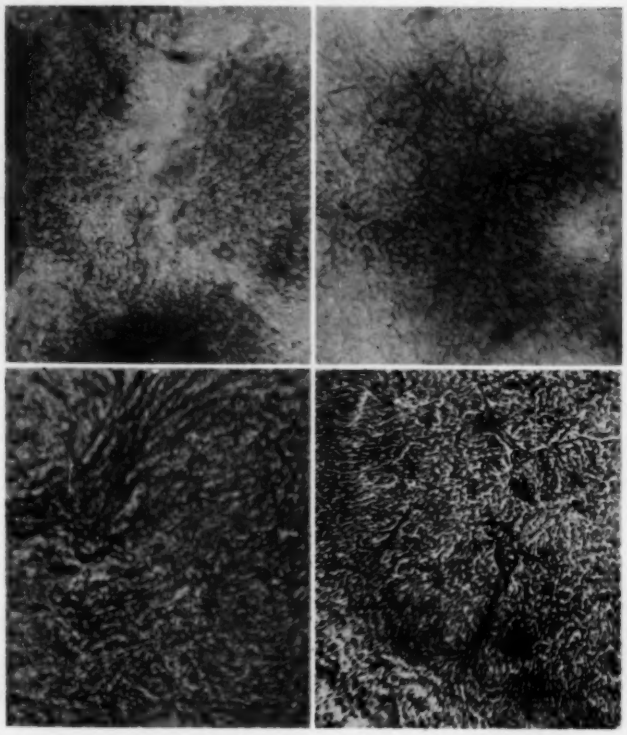
**ETIOLOGIC AGENT:**
Actinomyces israeli (anaerobic)

**CLINICAL:**



Chronic superficial and visceral granulomatous lesions which break down and form multiple sinuses.

**MICROSCOPIC EXAMINATION:**

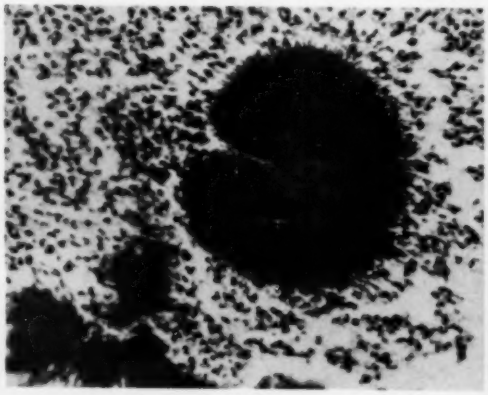


Direct: "Sulfur granules" in exudate; gram-positive branching elements. May or may not have clubbed ends.

**CULTURES:**

Colony: Grows anaerobicly below the surface of liquid or semi-solid medium; fuzzy, lobulated.
Microscopic: Tangled masses or fragments of delicately branched hyphae. (Bacteria-like.)

**HISTOPATHOLOGY:**



Granuloma: Characteristic "granules" found in cellular exudate.

**TREATMENT:**

Surgical excision.
Medical: Penicillin; sulfadiazine; chlortetracycline; chloramphenicol; streptomycin.

**DISTRIBUTION:** World wide.

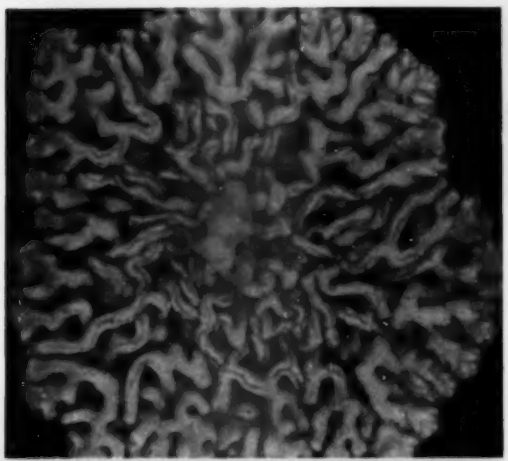
# NOCARDIOSIS

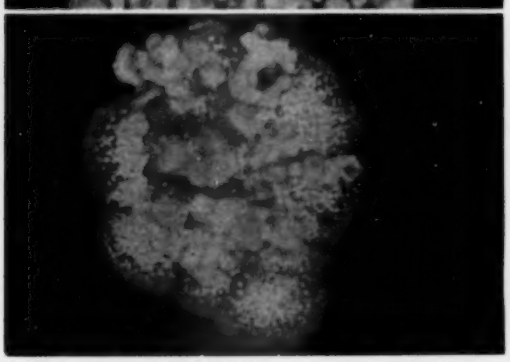
**ETIOLOGIC AGENT:**
Nocardia asteroides (aerobic).

**CLINICAL:**

Chronic pulmonary or systemic disease characterized by visceral granulomatous lesions.

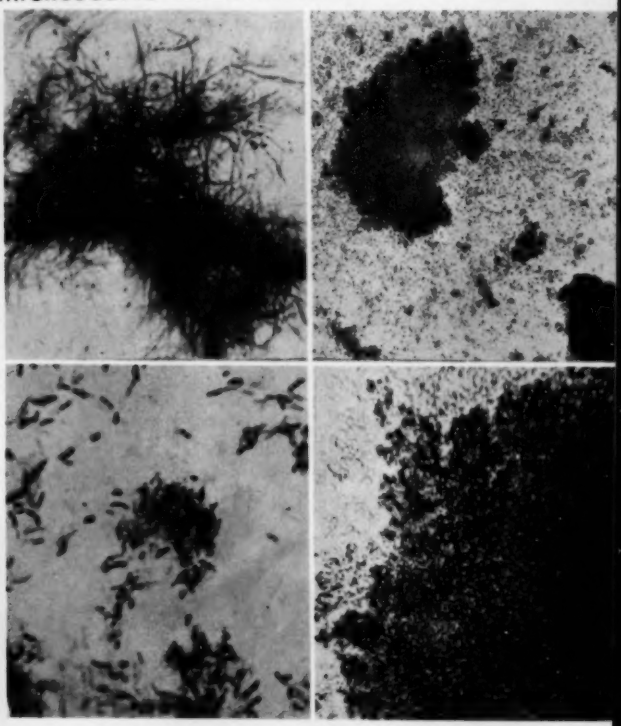
**CULTURES:**





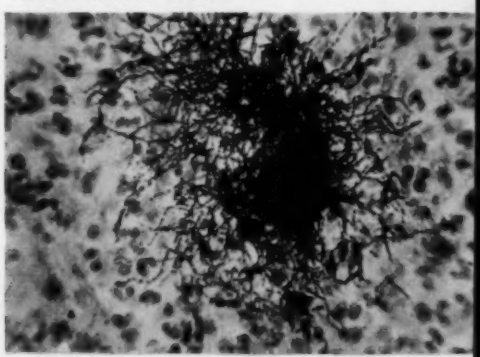
Colony: Sabouraud's medium: Glabrous, irregular, folded, raised. May be granular or powdery. Varies from white to yellow-tan. Microscopic: Delicate branched intertwining filaments. Gram positive. Acid fast.

**MICROSCOPIC EXAMINATION:**



Direct: Intertwining branching mycelial fragments (bacteria-like). Gram positive. No clubs formed on ends of hyphae. Nocardia asteroides is acid fast.

**HISTOPATHOLOGY:**



Granuloma. Branching filaments. Gram positive. may be acid fast.

**TREATMENT:**

Penicillin; sulfadiazine; chlortetracycline.

**DISTRIBUTION:** World wide.

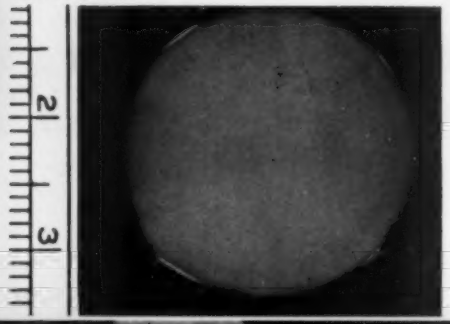
# CRYPTOCOCCOSIS

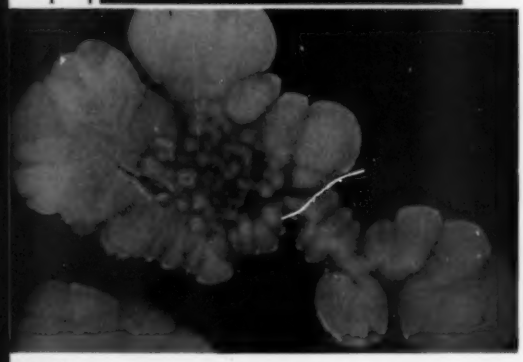
**ETIOLOGIC AGENT:**
Cryptococcus neoformans.

**CLINICAL:**

Subacute or chronic infection involving lungs, skin, brain meninges, and other organs.

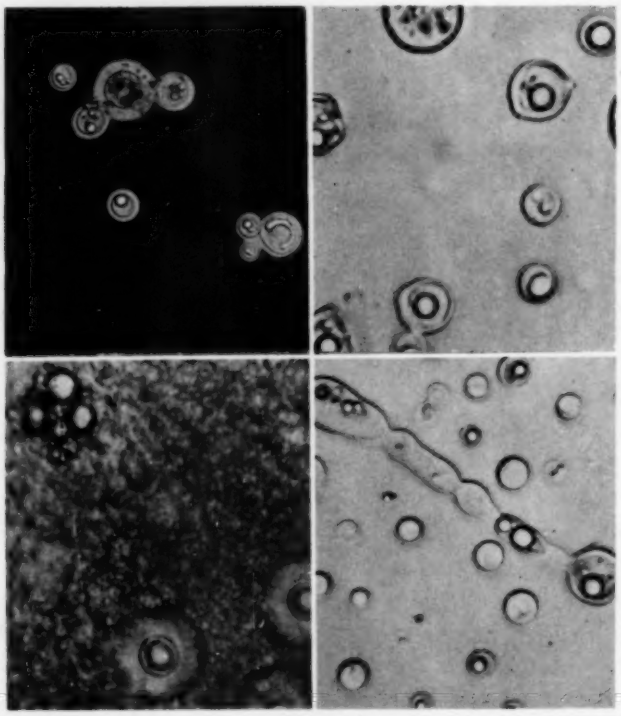
**CULTURES:**



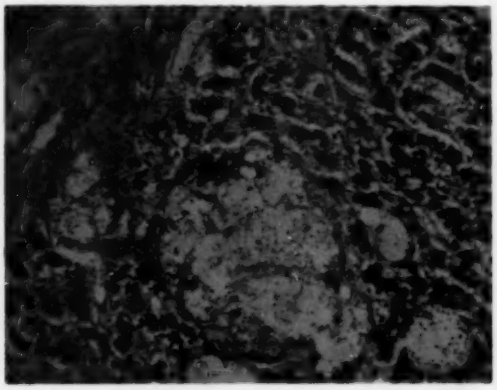
Colony:
Sabouraud's medium: Colonies white, cream to brown, yeast-like at all temperatures.
Littman's oxgall medium: Yeast-like, light purple; surface moist; shiny; center heaped.
Microscopic: Budding cells and occasional organisms with short germ tubes. May or may not have capsule. Older cultures show only budding forms—capsule prominent.

**MICROSCOPIC EXAMINATION:**



Direct: Thin walled, ovoid to spherical, single or budding yeast-like organisms; surrounded by wide refractile gelatinous capsule best demonstrated in india ink preparation.

**HISTOPATHOLOGY:**



Chronic inflammatory reaction; macrophages and giant cells may be present; often numerous single or budding organisms; cyst formation frequent.

**TREATMENT:** Resection. Actidione. Sulfonamides.

**DISTRIBUTION:** World wide.

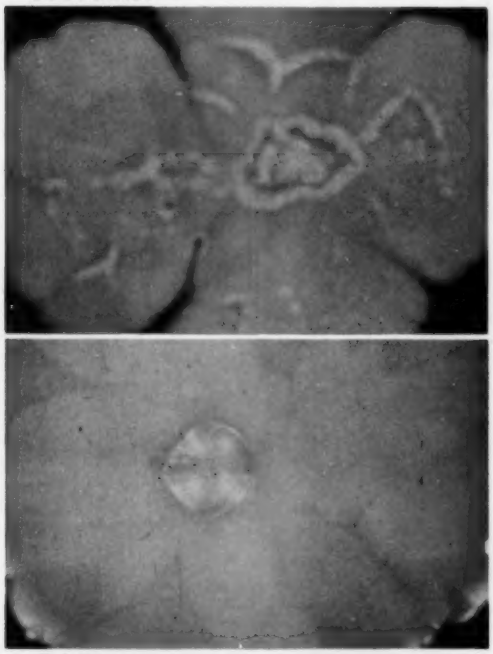
# MONILIASIS

**ETIOLOGIC AGENT:**
Candida albicans.

**CLINICAL:**

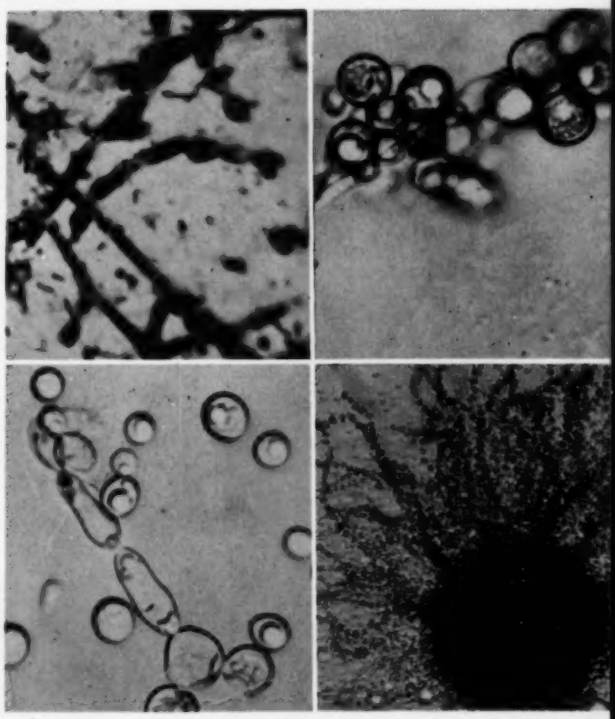
Acute or subacute infections of vagina, hands, mouth, lungs; occasionally a septicemia or meningitis.

**CULTURES:**



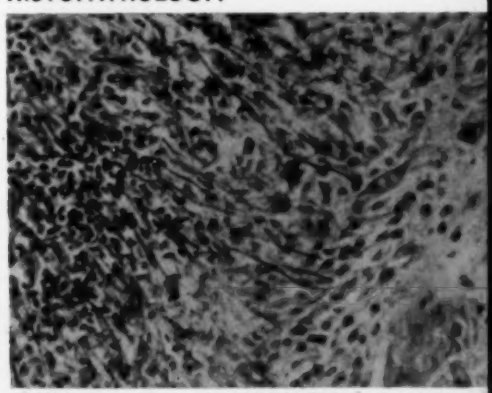
Colony:
Sabouraud's medium: Cream colored, yeast-like; distinct yeasty odor.
Littman's oxgall medium: Light purple, yeast-like, dry, waxy.
Microscopic: Small budding oval yeast-like cells. Pseudohyphae. Large chlamydospores characteristic of C. albicans on corn meal or chlamydospore agar.

**MICROSCOPIC EXAMINATION:**



Direct: Small oval budding yeast-like cells—occasional pseudohyphae.

**HISTOPATHOLOGY:**



Mild to severe chronic inflammatory reaction.
Round or oval, single or budding organisms and pseudomycelial fragments.

**TREATMENT:**

Gentian violet for cutaneous lesions. Alkaline douches for vaginitis. Iodides. $KMnO_4$ soaks, etc.

**DISTRIBUTION:** World wide.

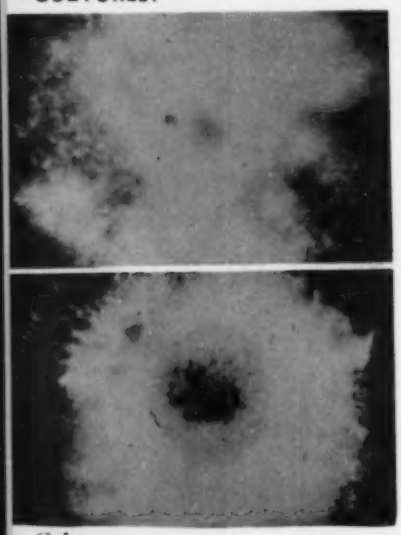
# NORTH AMERICAN BLASTOMYCOSIS

**ETIOLOGIC AGENT:**
Blastomyces dermatitidis.

**CLINICAL:**

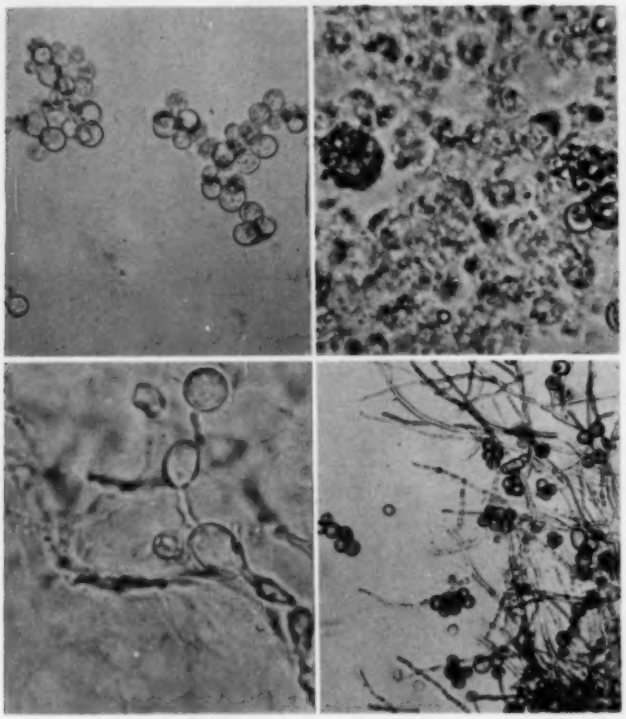
Chronic infection—granulomatous and suppurative. Mainly in skin, lungs, and bones.

**CULTURES:**



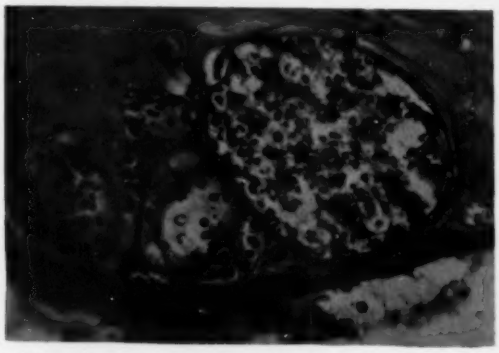
Colony:
Sabouraud's medium: Filamentous, white at 20 C. Yeast-like, white at 37 C.
Littman's oxgall medium: Umbonate with isolated tufts of mycelium, slightly furrowed, light purple at 20 C. Yeast-like purple at 37 C.
Microscopic: Septate hyphae with single small conidia. At 37 C. cells are double contoured and yeast-like with single buds from parent cell.

**MICROSCOPIC EXAMINATION:**



Direct: Budding cells—thick double wall. Refractile. Single buds. No hyphae present.

**HISTOLOGY:**



Granulomatous lesions in skin and viscera containing double-walled single and budding organisms.

**TREATMENT:**

Stilbamidine (i. v.). Surgical resection. X-rays. Vaccines.

**DISTRIBUTION:** United States, Canada, and England.

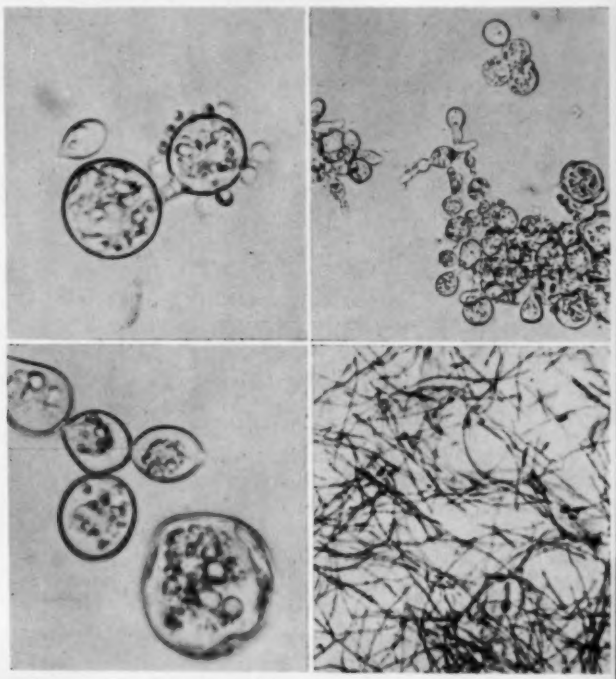
# SOUTH AMERICAN BLASTOMYCOSIS

**ETIOLOGIC AGENT:**
Blastomyces brasiliensis.

**CLINICAL:**

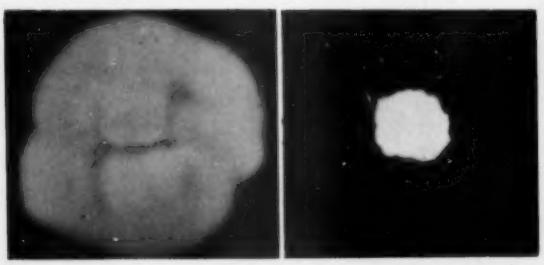
Chronic granulomatous lesions of skin, mucous membranes, lymph nodes, and internal organs.

**MICROSCOPIC EXAMINATION:**



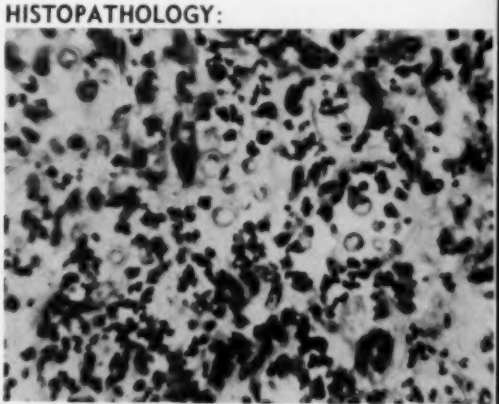
Direct: Single and multiple budding thick-walled yeast-like cells, 10-60 $\mu$ in diameter. Single cells similar to Blastomyces dermatitidis.

**CULTURES:**



Colony:
Sabouraud's medium: Filamentous, white at 20 C. Yeast-like, cream to white at 37 C.
Littman's oxgall medium: Slow-growing, capitate, furrowed, fuzzy, absorbs dye from medium. Yeast-like light purple at 37 C.
Microscopic: Septate hyphae with small single conidia at 20 C. Parent cell has multiple buds in yeast-like colony at 37 C.

**HISTOPATHOLOGY:**



Granulomatous reaction with giant cells in all tissue. Organisms present may be single or multiple and yeast-like; multiple budding organisms are characteristic.

**TREATMENT:** Sulfonamides. Iodides. Vaccine.

**DISTRIBUTION:** South America.

cell.

**DISTRIBUTION:** United States, Canada, and England.

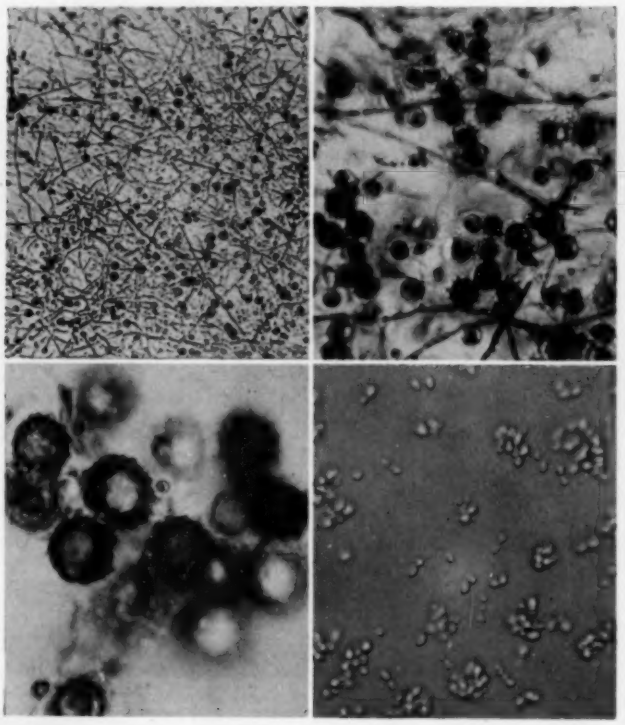
# HISTOPLASMOSIS

**ETIOLOGIC AGENT:**
Histoplasma capsulatum.

**CLINICAL:**

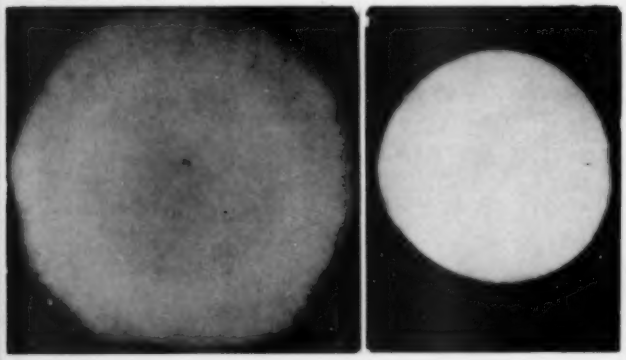
May be asymptomatic pulmonary, symptomatic pulmonary, localized, or disseminated (nonfatal or fatal). Severe infection characterized by emaciation, leukopenia, anemia, and irregular pyrexia. Frequent lymphadenopathy, splenomegaly, hepatomegaly, and ulceration of naso-oral-pharyngeal cavities and intestines.

**MICROSCOPIC EXAMINATION:**



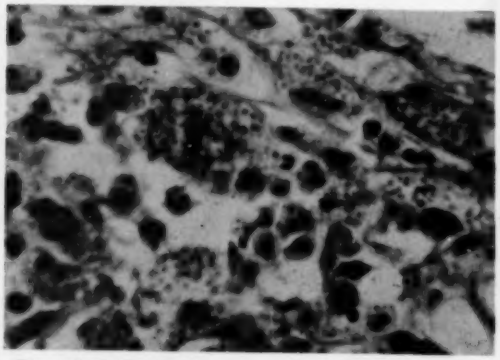
Direct: Small (1-5 $\mu$) oval yeast-like cells in macrophages or other reticulo-endothelial cells of bone marrow, lymph nodes, spleen, liver, blood, etc.

**CULTURES:**



Colony:
Sabouraud's medium: Cottony white aerial mycelium at 20 C. is tan and yeast-like.
Littman's oxgall medium: Slow growing, capitate, furrowed, white to purple at 20 C.; light purple and yeast-like at 37 C.
Microscopic: Branching septate hyphae with lateral or sessile, round or oval conidia. Old colonies have characteristic round to pyriform tuberculate chlamydospores (7-15 $\mu$). At 37 C. organisms are small, round or oval, budding yeast-like cells.

**HISTOPATHOLOGY:**



Chronic inflammatory reaction. Small, round or oval, single or budding organisms in macrophages, giant cells or reticulo-endothelial cells.

**DISTRIBUTION:**

Western Hemisphere. England. South Africa. Java.

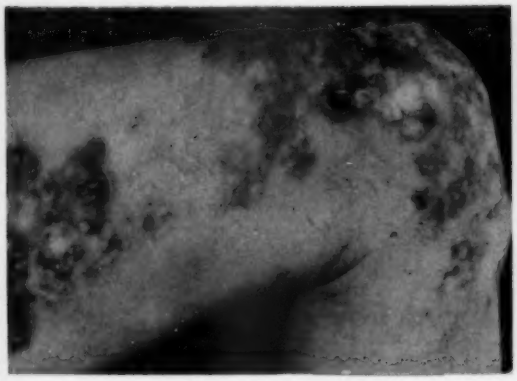
**TREATMENT:**

Symptomatic therapy, surgical excision, quinacrine, ethyl vanillate, and promine.

# SPOROTRICHOSIS

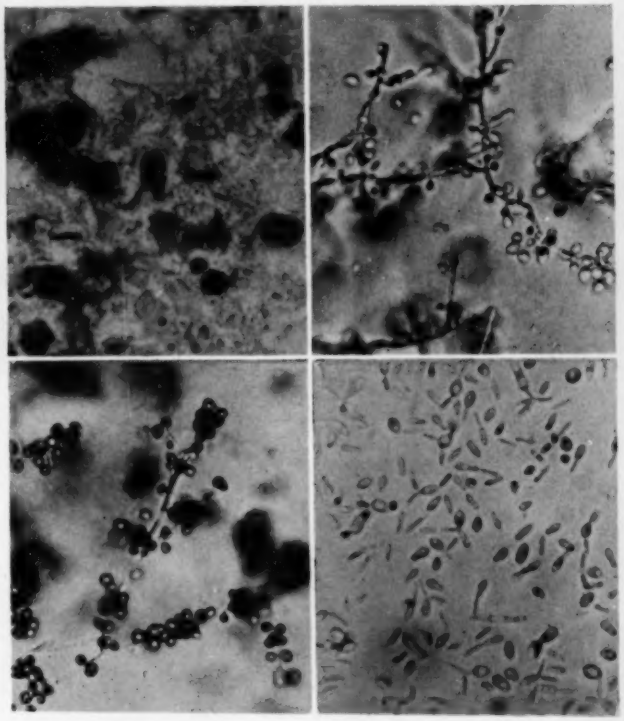
**ETIOLOGIC AGENT:**
Sporotrichum schenckii.

**CLINICAL:**



Chronic infection with nodular lesions of lymph nodes, skin, or subcutaneous tissues, which soften and break down to form indolent ulcers. Occasionally disseminated form—acute and rapidly fulminating.

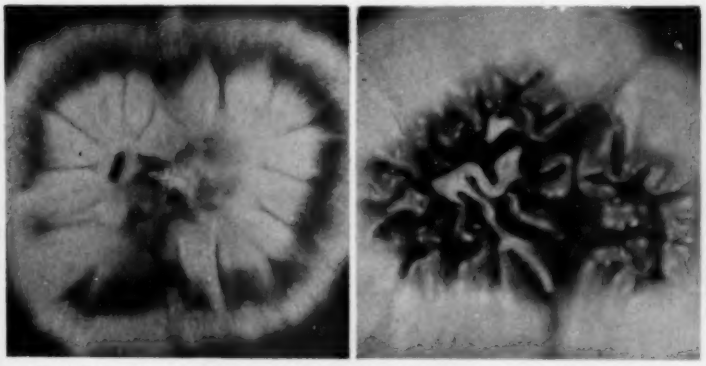
**MICROSCOPIC EXAMINATION:**



Direct: Gram-positive, cigar-shaped bodies within polys or giant cells. Very difficult to find. Culture necessary for diagnosis.

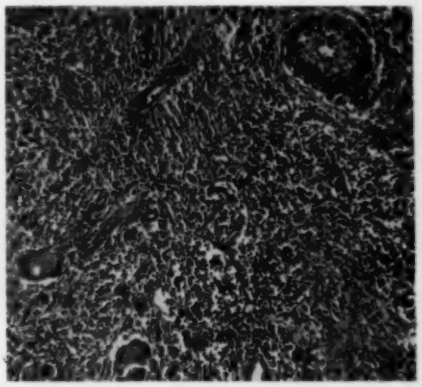
**CULTURES:**

Colony:
Sabouraud's medium: Small, flat, or umbonate, no aerial mycelia. Later moist wrinkled, and membranous. Color cream to white, darkens brown to black with age. Yeast-like and cream colored at 37 C.
Littman's oxgall medium: Small, corrugated; center dry, deep purple.



Periphery finely furrowed, moist, and light purple. Yeast-like and light purple at 37 C.
Microscopic:
Branching septate hyphae bearing pyriform to oval conidia in clusters from lateral branches.

**HISTOPATHOLOGY:**



Granulomatous reaction with giant cells and macrophages. Organisms: extremely difficult to demonstrate. Diagnosis: made by culture.

**TREATMENT:** K. I.; stilbamidine; x-ray; surgery.

**DISTRIBUTION:**

North America. Europe. South Africa.

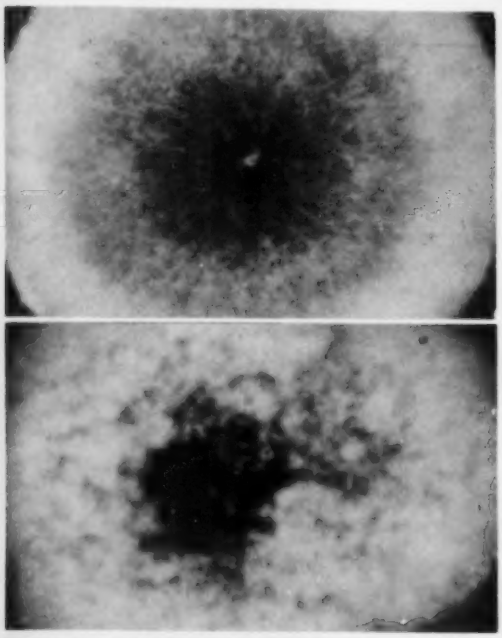
# COCCIDIOIDOMYCOSIS

**ETIOLOGIC AGENT:**
Coccidioides immitis.

**CLINICAL:**

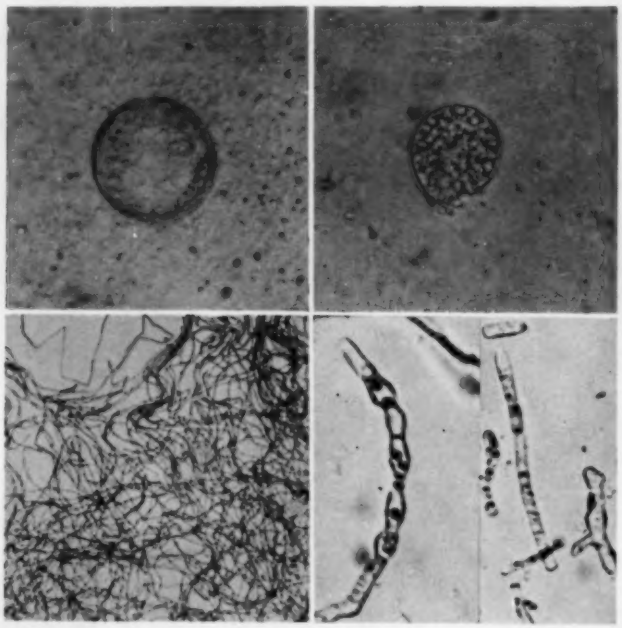
Primary—acute but benign, self-limited respiratory disease. Progressive — chronic malignant, disseminated, involving cutaneous, subcutaneous, visceral, and bone tissue. (Dark-skinned races.) Residual pulmonary —single cyst or single lobe involved.

**CULTURES:**



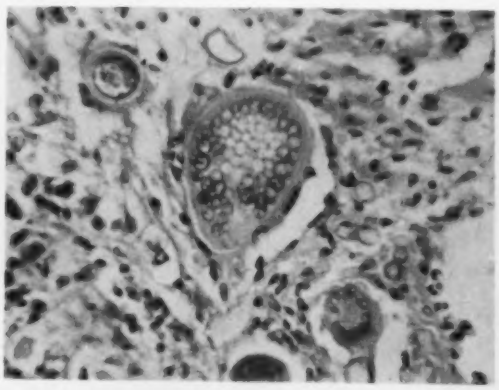
Highly infectious; extreme caution should be exercised when handling cultures.
Colony:
Sabouraud's medium: Moist—then cottony; white, then brown. Very friable, and spores spread easily.
Littman's oxgall medium: Slower growing, white, heaped and somewhat matted, then cottony. Border irregular.
Microscopic: Branching septate hyphae that break up forming thick-walled, pillow-shaped or rectangular arthrospores.

**MICROSCOPIC EXAMINATION:**



Direct: Nonbudding, spherical, thick-walled structure, 20-80 $\mu$ in diameter, filled with numerous endospores (2-5 $\mu$). Immature spherules may be single nonbudding form.

**HISTOPATHOLOGY:**



Chronic granuloma and abscess formation with macrophages and giant cells. Large spherules filled with endospores and single spores are generally prominent.

**TREATMENT:** Surgical resection.

**DISTRIBUTION:** United States. South America?

# CHROMOMYCOSIS

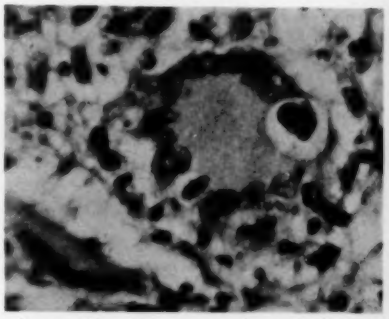
**ETIOLOGIC AGENTS:**

(1) Phialophora verrucosa; (2) Hormodendrum pedrosoi; (3) Hormodendrum compactum; (4) Hormodendrum sp.; (5) Cladosporium sp.

**CLINICAL:**

Warty cutaneous nodules; slow-growing, papillomatous vegetations which may or may not ulcerate. May occur on hands, ear, neck, chest, shoulders, or buttocks, but generally confined to head and extremities. Occasionally brain abscesses.

**HISTOPATHOLOGY:**

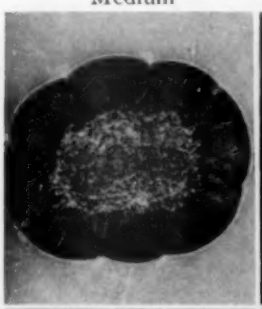
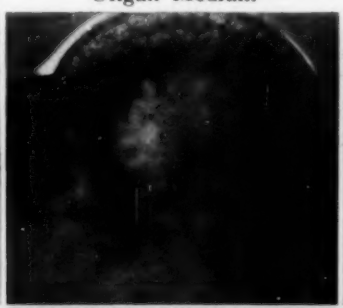
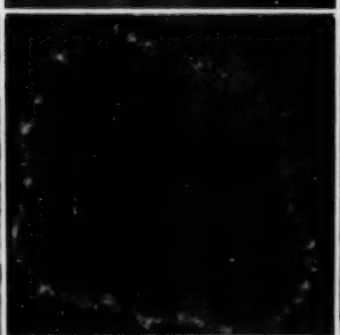
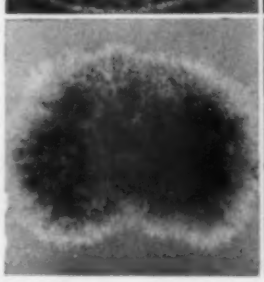


Granuloma formation with numerous giant cells. Dark brown, single or clustered, round or oval organisms showing division by fission. May be located in giant cells or surrounded by macrophages.

**DIRECT MICROSCOPIC EXAMINATION:**

Organisms small, single or clustered, round, thick-walled, brown bodies. Multiply by fission or splitting. Cultures necessary for identification of species.

**CULTURES:**

| | Sabouraud's Medium | Littman's Oxgall Medium | Microscopic Characteristics | |
|---|---|---|---|---|
| Phialophora verrucosa: |  |  | | |
| Hormodendrum pedrosoi: | |  | | |
| Hormodendrum compactum: |  | | | |

**TREATMENT:**

Surgical excision. Cauterization. Iodides.

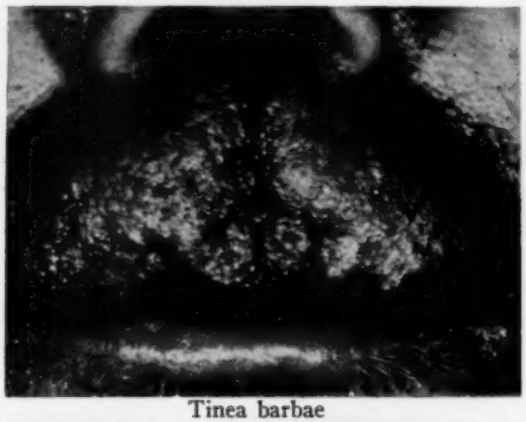
**DISTRIBUTION:** World wide.

# DERMATOMYCOSIS

**ETIOLOGIC AGENTS:**
Trichophyton sp., Microsporum sp., and Epidermophyton sp. (See following two panels.)

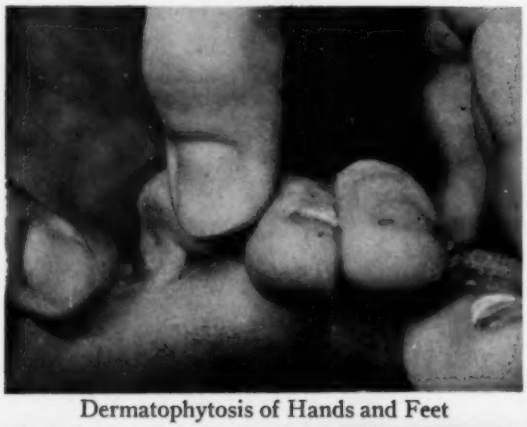
**CLINICAL DISEASE:**
Chronic lesions of skin, nails and hairs varying from simple scaling to deep granulomatous and kerion-like tumors.

Tinea capitis



Tinea barbae

Tinea corporis



Dermatophytosis of Hands and Feet

Tinea cruris

Tinea ungium

**HISTOPATHOLOGY:**
Septate mycelia growing in epidermis and adjacent structures often associated with varying amounts of secondary bacterial infection.

**TREATMENT:**
Fungicides. Epilation. X-rays. Personal hygiene.

**DISTRIBUTION:** World wide.

DERMATOMYCOSIS

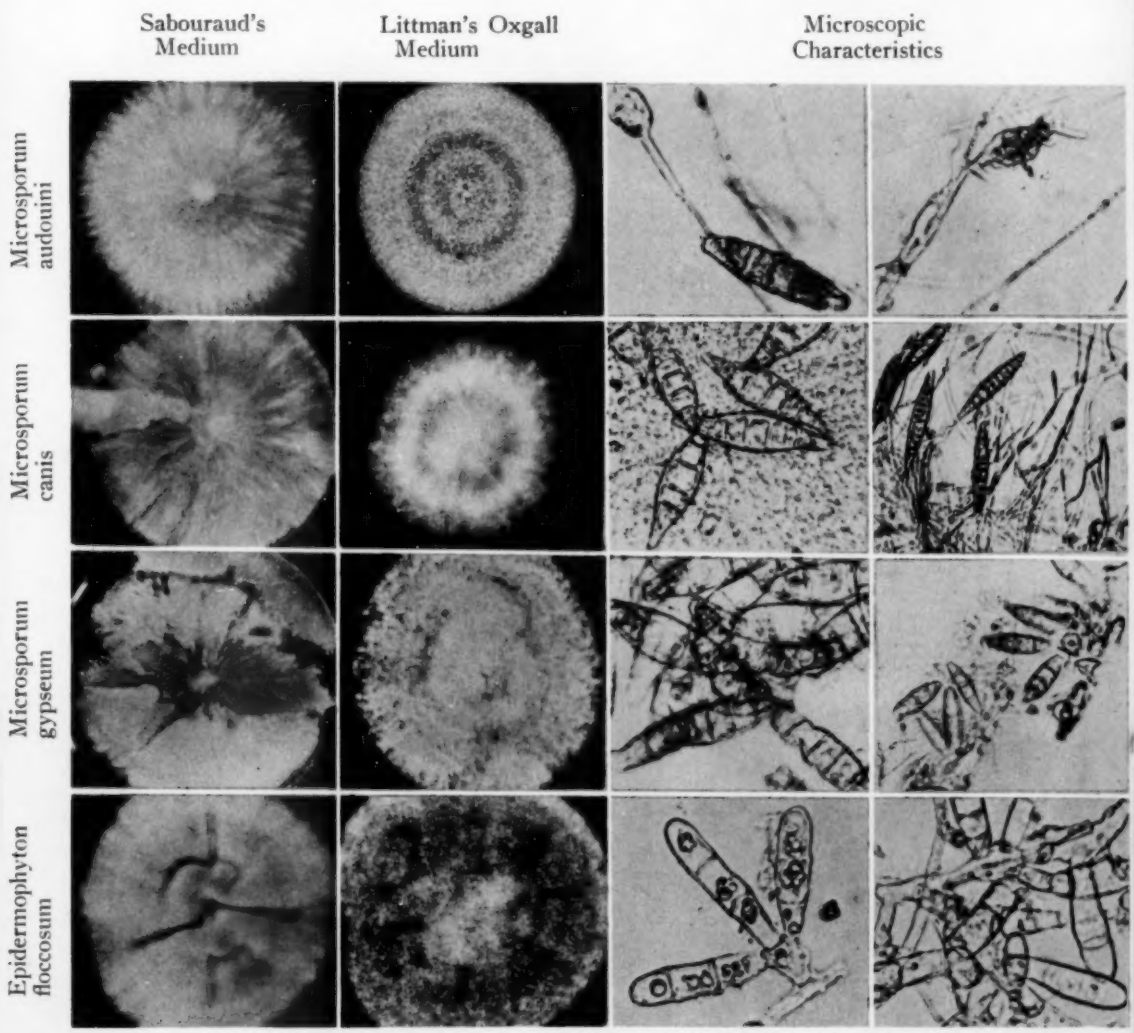
# EPIDERMOPHYTON & MICROSPORUM SP.

**ETIOLOGIC AGENTS:**
Microsporum audouini; Microsporum canis; Microsporum gypseum; Epidermophyton floccosum.

**CLINICAL DISEASE:**
Often the causative agent in tinea capitis and tinea cruris and occasionally in the other dermatomycoses.

**DIRECT MICROSCOPIC EXAMINATION:**
Fragments of mycelia and arthrospores in scales of epidermis and hairs. Identification of etiologic agent depends on cultural characteristics.

**CULTURES:**



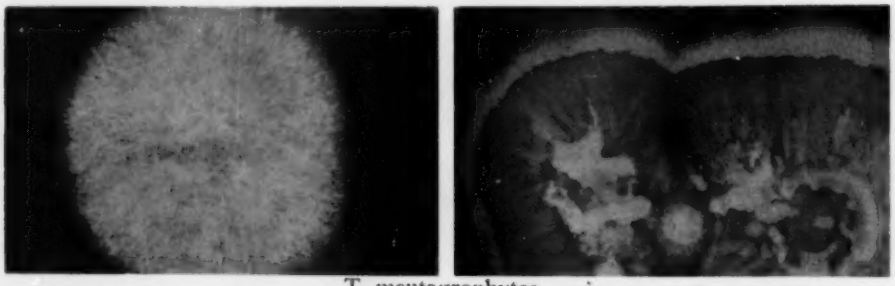
# DERMATOMYCOSIS

**ETIOLOGIC AGENT:**
Trichophyton sp.

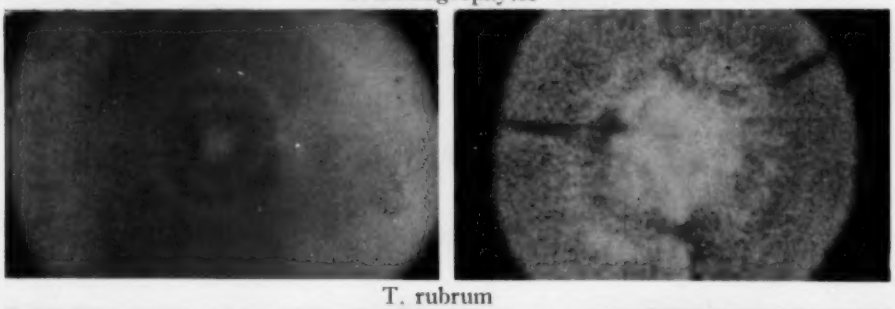
**CULTURES:**

Cultural Characteristics

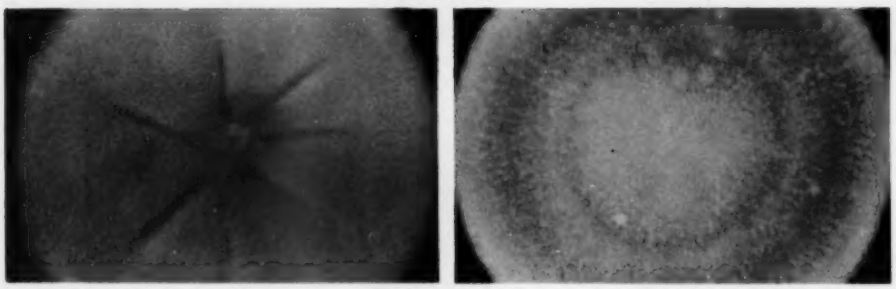
Sabouraud's Medium | Littman's Oxgall Medium



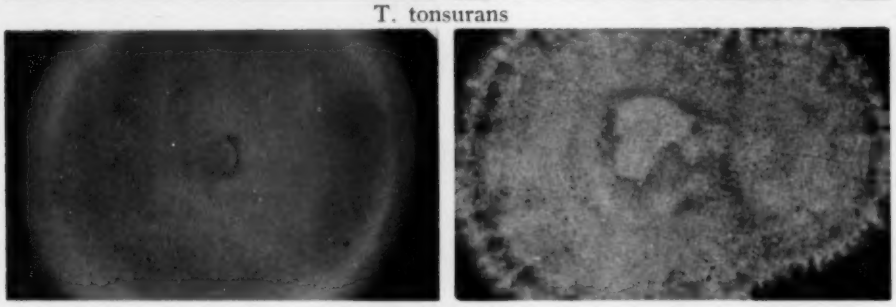
T. mentagrophytes



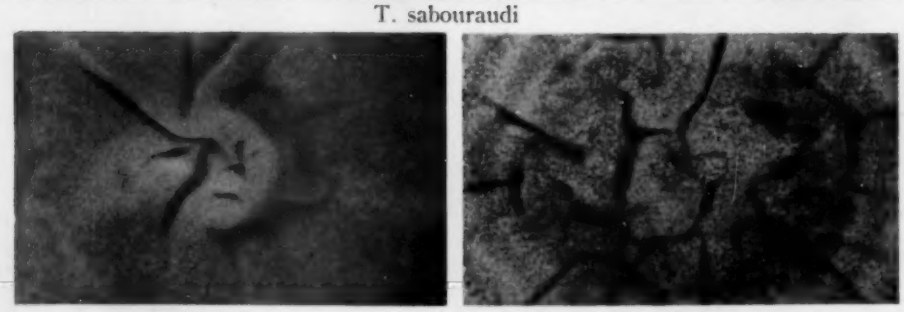
T. rubrum



T. tonsurans

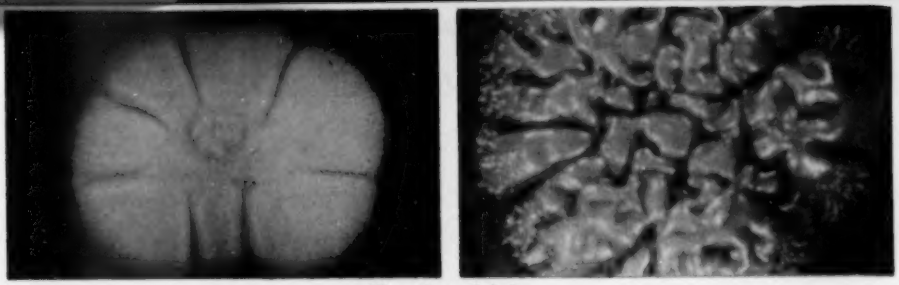


T. sabouraudi

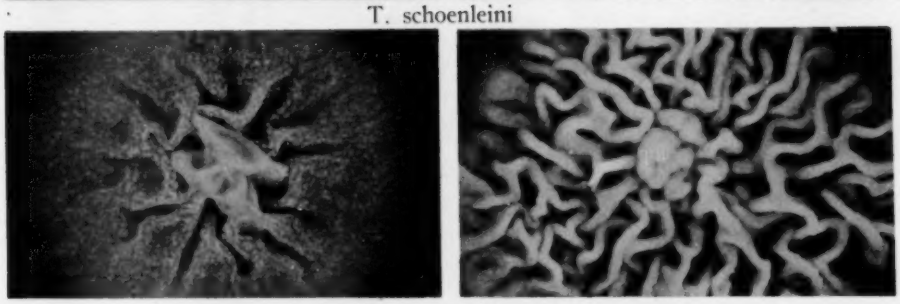


T. sulfureum

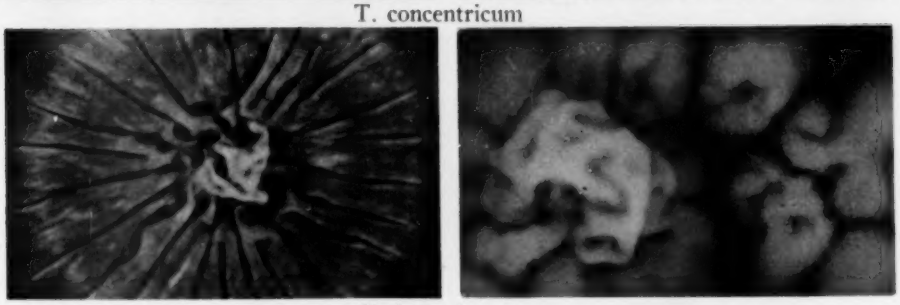
# OCCASIONAL



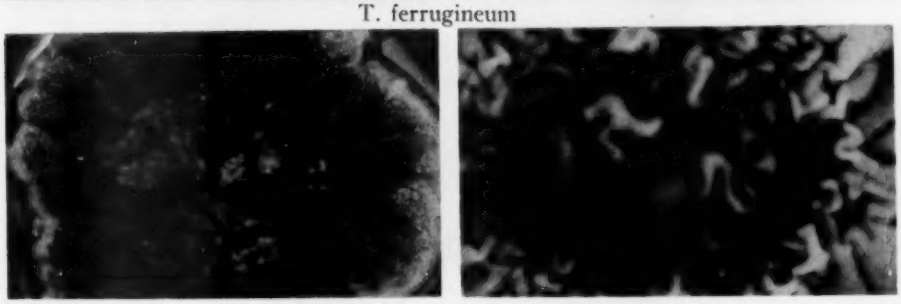
T. schoenleini



T. concentricum



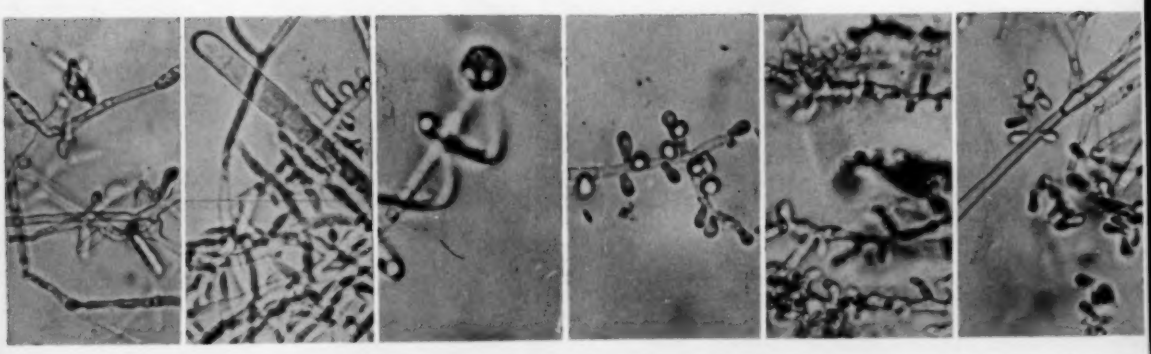
T. ferrugineum



T. violaceum

**DIRECT MICROSCOPIC EXAMINATION:**

Fragments of septate mycelia and arthrospores in scales of epidermis, fragments of nails and hairs. Identification of etiologic agent depends on cultural characteristics.



T. sulfureum

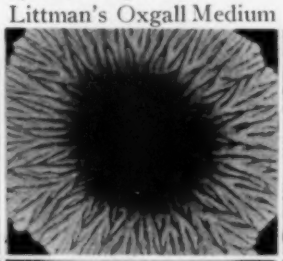
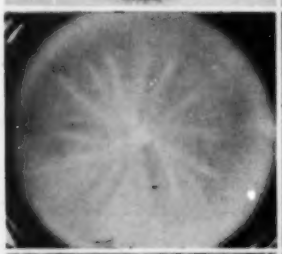
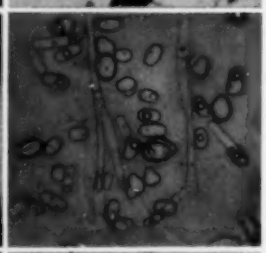
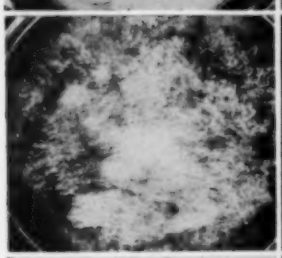
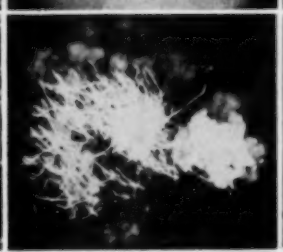
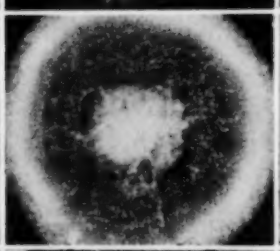
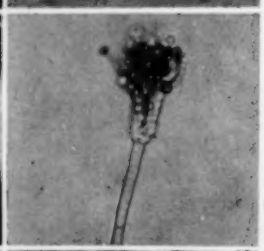
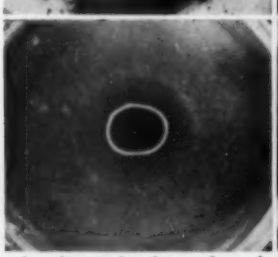
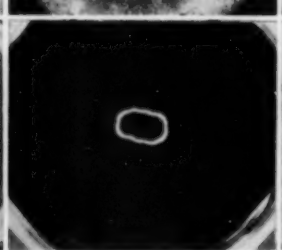
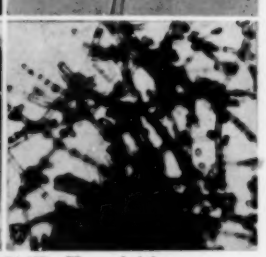
# OCCASIONAL PATHOGENS

**ETIOLOGICAL AGENTS:** Aspergillus sp.; Geotrichum sp.; Mucor sp.; Penicillium sp.; Hormodendrum sp.

**CLINICAL DISEASES:** Occasionally lesions like otomycosis, pneumonia, diarrhea, and brain abscesses are produced by these fungi generally considered to be contaminants. No other etiologic agents can be isolated.

**DIRECT MICROSCOPIC EXAMINATION:**
The material examined contains septate hyphae and occasional spores. Mucor sp. mycelia are non-septate. Material must be cultured for specific diagnosis.

**CULTURES:**

| | Sabouraud's Medium | Littman's Oxgall Medium | Microscopic Characteristics |
|---|---|---|---|
| Aspergillus sp. | |  | |
| Geotrichum sp. |  | |  |
| Mucor sp. |  |  | |
| Penicillium sp. | |  |  |
| Hormodendrum sp. |  |  |  |

**HISTOPATHOLOGY:** Reaction in tissue is that of a chronic granuloma containing fragments of mycelia and occasional spores. Material must be cultured for specific identification.

**TREATMENT:** Fungicides.
Surgical excision.
Symptomatic care.

**DISTRIBUTION:** World wide.

# News and Comment

**South Central Pathologists Meet.**—The South Central Region College of American Pathologists and the Louisiana Pathology Society will hold a joint meeting at the Jung Hotel in New Orleans, Feb. 24-26, 1955. The program will include a symposium on "Leprosy" at the National Leprosarium, at Carville, La., moderated by Dr. Lawrence L. Swan, Pathologist, U. S. Public Health Service Hospital, New Orleans and Carville, and one on "Legal Medicine," moderated by Dr. Charles P. Larson, Pathologist, Tacoma General Hospital, Tacoma, Wash. Further information may be obtained from Dr. Albert L. McQuown, Program Chairman, Charity Hospital of Louisiana, New Orleans 12.

**Reorganization of Commissioned Reserve.**—A major expansion and reorganization of the Commissioned Reserve of the Public Health Service, Department of Health, Education, and Welfare, as a national defense measure has been announced by Surgeon General Leonard A. Scheele. The Service expects to commission an additional 2,000 reserve officers by June 30, 1955, and present plans call for the commissioning of another 3,000 officers during the 1955-1956 fiscal year.

The Service has been assigned extensive new defense responsibilities as the result of a delegation by the Federal Civil Defense Administration to the Department of Health, Education, and Welfare recently approved by the President. In addition to building up the Commissioned Reserve to emergency strength, the Service is stepping up research in disaster health problems and is developing a program to reinforce state and local health departments in time of national crisis.

In strengthening the Commissioned Reserve, the Service has two fundamental objectives: (1) effective emergency of professional personnel with training and experience in public health and (2) augmenting these forces with physicians and other professional people not normally working in the field of public health.

Dr. Scheele said that an officer of the emergency reserve would be called to active duty without his consent only in the event of a national emergency publicly recognized as requiring such action and that an officer of the emergency reserve already performing important health functions would not be called for service in another area unless the situation in that area clearly justified it. Officers in the emergency reserve may request active duty at any time and will be considered for available assignments.

The emphasis initially will be on the commissioning of physicians, dentists, sanitary engineers, and nurses, particularly physicians, the Service said.

In the near future, the Public Health Service expects to start training officers of the emergency reserve in the health problems associated with atomic, biological, and chemical warfare and other national emergencies. As the expansion goes forward, the Service hopes to train all reserve officers who can arrange to be away from their regular work for brief periods.

**Appointment of Dr. Eugene T. Standley.**—Dr. Eugene T. Standley has been appointed assistant professor of pathology at the University of Missouri School of Medicine. Dr. Standley was formerly instructor in pathology at Columbia University and assistant pathologist at the Presbyterian Hospital, New York.

**Annual Harrison S. Martland Lecture.**—The 20th annual Harrison S. Martland Lecture was given by Dr. Fred W. Stewart of the Memorial Center for Cancer and Allied Diseases, New York, on Dec. 8, 1954, at the Academy of Medicine of New Jersey, in Newark. Dr. Stewart discussed "Osteogenic Sarcoma Caused by Therapeutic Irradiation."

**Appointment of Dr. Leland Stoddard.**—Dr. Leland Stoddard, formerly of Duke University School of Medicine, has been named chairman of the Department of Pathology of the University of Georgia School of Medicine.

**Cancer Seminar.**—The Third Annual Cancer Seminar of the Arizona Division of the American Cancer Society was presented at Paradise Inn in Phoenix, Ariz., on Jan. 13, 14, and 15. The program was concentrated this year on Cancer of the Gastrointestinal Tract, Female Genital Tract, Genitourinary Tract, and Bone Tumors. An Evaluation of Present-Day Treatment for Cancer of the Breast was presented, as well as a discussion of the American Cancer Society's study of Smoking Practices in Relation to Health and Cancer.

**Research Potential in Pathology.**—Recognizing that a shortage exists in training investigators, a symposium was held on "Increasing the Research Potential in Pathology" in Bethesda, Md., in January, 1954, under the auspices of the Pathology Study Section of the National Institutes of Health. The problems in recruitment, training, post-training career, finances, and other aspects were considered. No facile remedies were forthcoming, but it was apparent that many things can be done to improve the situation. The recommendations and a full report of the papers and discussion were published in *Laboratory Investigation* **3**:377-448 (Sept.-Oct.) 1954. All pathologists have a stake in this problem and should find these proceedings of interest.

**Meeting of Histochemical Society.**—The Histochemical Society will meet in Philadelphia, April 4-5, 1955, at Jefferson Medical College of Philadelphia. A symposium on "Histochemistry and the Elucidation of Kidney Structure and Function" will be held Tuesday afternoon, April 5. Hotel headquarters will be at the Benjamin Franklin.

# Books

**Clinical Pathological Conferences of Cook County Hospital: Volume I; Cardiovascular-Renal Problems.** Edited by Hans Popper, M.D., Ph.D., and D. S. Kushner, M.D. Price, $5.00. Pp. 325, with 65 illustrations. Blakiston Company, 575 Madison Ave., New York 22, 1954.

Patterned in part after the "Cornell Conferences on Therapy" in internal medicine, the present volume is the first of a projected series derived from the clinical-pathological conferences at Cook County Hospital. This volume covers cardiovascular and renal problems presented at conferences in recent years. Eleven cardiac problems are considered, ranging from nutritional myocardosis to tuberculous pericarditis. Lupus erythematosus, periarteritis, and scleroderma are included in the cases showing primarily vascular lesions. The eight renal problems include eclampsia, nephrosclerosis, and lower nephron nephrosis.

The format of the volume is that of the conference with the remarks of the participants edited to provide continuity. The cases include both gross and microscopic illustrations of pertinent pathological material. A short list of references has been included for each case, and in some cases editorial comments have been added. Representative clinical problems have been selected, and the major discussion centers about the field in question. However, solution of each case depends upon recognition of an unusual aspect of the disease. Thus, while volumes of this type sacrifice to some degree the logical presentation of textbooks, the spontaneity and challenge of the conference is retained. As a stimulus to the imagination and perspective of internist and pathologist alike, this first volume of the series will prove valuable.

**A.M.A. Fundamentals of Anesthesia.** Edited by the Consultant Committee for Revision of Fundamentals of Anesthesia, a Publication of the Council on Pharmacy and Chemistry of the American Medical Association. Third Edition. Price, $5.00. Pp. 279, with 89 illustrations. W. B. Saunders Company, 218 Washington Sq., Philadelphia 5; W. B. Saunders Company, Ltd., 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1954.

The first edition of this book came out in 1942 to meet the wartime needs for trained anesthetists. Its acceptance was immediate. In 1944 a second edition was published, and the supply of two printings was soon exhausted. In the ensuing 10 years the work has come to be looked upon as the textbook for medical student and resident instruction. The demands for the book and the many new developments in anesthesia made a revised edition mandatory. This task has been well done by the committee appointed by the Council on Pharmacy and Chemistry of the American Medical Association.

The book leads the reader through an introduction of the principles of the basic sciences related to anesthesia, a section which is much more comprehensive than in previous editions. The rest of the book is concerned with clinical aspects. It deals adequately with general and regional anesthesia, preoperative and postoperative care, and the treatment of complications. There is a special section dealing with obstetric, pediatric, and geriatric anesthesia. The clinical methods presented are practical and sound, and basic principles for a safe anesthesia are printed in bold-face type throughout. The text is written in an outline style and well organized, facilitating quick reference. It is as up to date as possible, incorporating the latest drugs and techniques.

The work may be condemned by some as being of the cook book style or a quick course in anesthesia but is to be recommended for its complete, concise coverage of the essentials of clinical anesthesia.

**Clinical Chemistry in Practical Medicine.** By C. P. Stewart, M.Sc., Ph.D., and D. M. Dunlop, B.A., M.D., F.R.C.P. Fourth Edition. Price, $5.00. Pp. 320, with 27 figures. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1954.

This is the fourth edition of a book on the everyday application of biochemistry to the problems of clinical medicine. The order of chapters has been changed from that in the 1949 edition, giving a more logical sequence. The chapter on Salt and Water Metabolism has been changed to Water and Electrolyte Metabolism, and a short section has been added on potassium.

The material on hormonal abnormalities and nutritional deficiencies has been reworked and expanded into two new chapters. A section on electrophoresis of plasma proteins has been added to the chapter on Proteins of the Blood Plasma. The text has generally been revised and new illustrations have been added.

The subject material of this book has been selected on the basis of importance and usefulness to the practitioner, house physician, and senior student. An appendix contains descriptions of laboratory tests and tables of normal results with the variations in disease. The restricted range of material covered limits the usefulness of this book to the specific groups for which it was written.

**Practical Fluid Therapy in Pediatrics.** By Fontaine S. Hill, M.D. Price, $6.00. Pp. 275, with 20 figures. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1954.

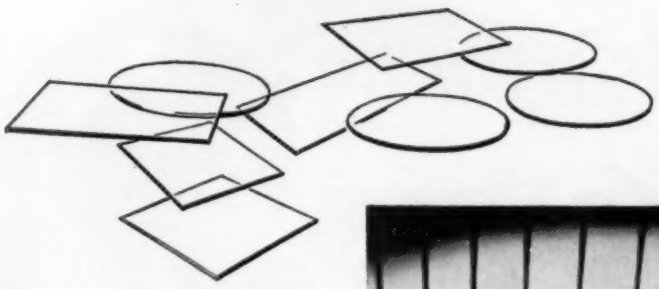
In this book the author has attempted to assemble for bedside application in pediatric patients the newer facts with reference to fluid and electrolyte therapy. In the section on acknowledgements he has a bibliography of recent contributions in this field, in which the names of Darrow, Gamble, Hartmann, Butler, Moyer, Newburgh, Smith, Pitts, and others testify to the up-to-date handling of the subject. The book is divided into three sections. Section 1 deals with basic physiologic principles and their clinical significance, with particular reference to the role of water in bodily economy, to normal electrolyte composition of bodily fluid, to abnormalities of electrolyte patterns, to renal regulation of fluid and electrolyte balance, and to the problems of dehydration and water intoxication. The second section deals with the diagnosis and treatment of clinical conditions and includes a discussion of solutions for parenteral administration and problems related to diarrhea, diabetic coma, renal disease, pyloric stenosis, acute infections, and other abnormal conditions. The third section deals with technical procedures relating to the obtaining of blood specimens and to parenteral fluid administration. There is an additional bibliography dealing with recent contributions to the subject.

The book is printed on high quality paper, and the illustrations are excellent. This book should be of value not only to pediatricians but also to all those interested in current problems of fluid balance and electrolyte therapy. No attempt has been made to enter into extensive discussions of these problems. In the words of the author, "The purpose of this book is to present basic principles of fluid and electrolyte therapy in such a manner that they may be readily employed at the bedside."

**Lectures on General Pathology.** By Dr. Florey and Nine Collaborators. Edited by Sir Howard Florey, Professor of Pathology, Sir William Dunn School of Pathology, University of Oxford. Price, $13.00. Pp. 733, with 343 illustrations. W. B. Saunders Company, 218 Washington Sq., Philadelphia 5; W. B. Saunders Company, Ltd., 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1954.

This volume grew out of lectures given by the authors to Oxford University medical students. The lectures do not form a complete survey of general pathology and therefore will not be suitable as an exclusive textbook in most medical schools of this country. However, the volume should be of value to the student or the teacher who is particularly interested in the principles of circulatory disturbances, inflammation, and immunity.

The lectures are well organized, well written, and well illustrated. They are particularly valuable for their attention to historical perspective and for their emphasis on contributions which experimental work has made to the understanding of disease processes. This approach should be stimulating to the beginning student. The use of this volume as supplementary reading during the medical student's introduction to pathology may help interest him in investigative work. It will certainly help him gain a clear understanding of the present status of man's knowledge of some important aspects of disease.



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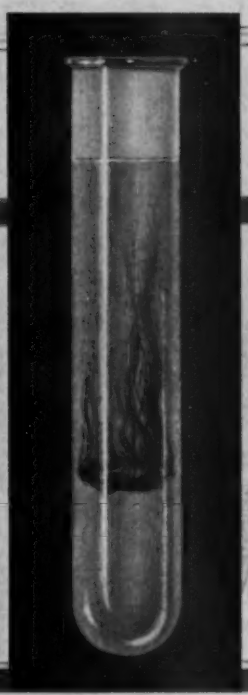

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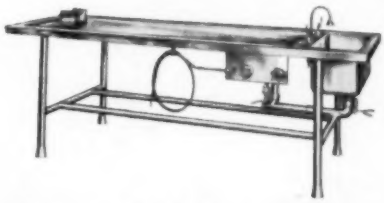

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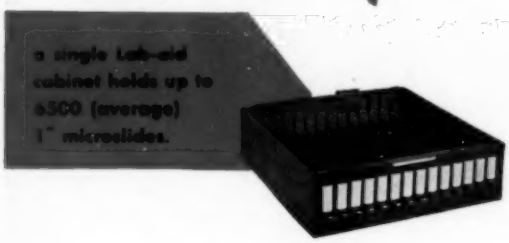


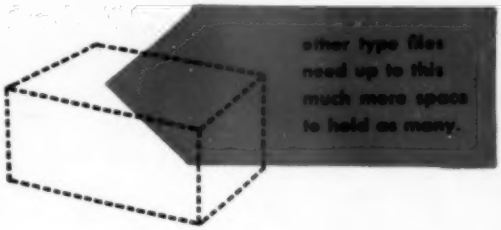


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